Director:
Patrick S. Fitze
Museo Nacional de Ciencias Naturales
Dpto. de Biodiversidad y Biología Evolutiva
Madrid, España

Instituto Pirenaico de Ecología
Jaca, España

University of Lausanne
Department of Ecology and Evolution
Lausanne, Switzerland

Tutor Académico:
José Luis Rubio
Universidad Autónoma de Madrid
Departamento de Ecología
Madrid, España

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Universidad Autónoma de Madrid
Facultad de Ciencias
Departamento de Ecología

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Tesis Doctoral
Virginia González Jimena

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CSIC
Madrid, 2013

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Facultad de Ciencias
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Stress Response in the Common Lizard *Lacerta vivipara* Jacquin, 1787

Memoria presentada por **Virginia González Jimena** para optar al Grado de Doctor

DIRECTOR:
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Para Vivi

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Implications of Corticosterone Effects on Behaviour

Table of Contents

Presentación

El concepto de estrés es algo complicado de definir y, sin embargo, todos nos hemos enfrentado al estrés en algún momento de nuestra vida y conocemos las consecuencias del mismo. En respuesta a un estimulo o situación potencialmente estresante se produce una respuesta en el organismo que conlleva una serie de cambios fisiológicos y conductuales que ayudan a lidiar con el estimulo o situación percibidos, aumentando las probabilidades de supervivencia inmediata. Esta respuesta fisiológica del organismo frente al estrés se traduce en un aumento en los niveles basales de glucocorticoides (cortisol o corticosterona dependiendo de la especie). La variación que puede producirse en los niveles basales de glucocorticoides no sólo está regulada por la percepción de un factor estresante, sino que a su vez sigue patrones estacionales y circadianos, depende de las características propias de cada individuo (sexo, edad, etc), e incluso de factores a nivel psicológico tales como la percepción individual sobre el control que tenga el individuo del estimulo causante de la respuesta al estrés. Además, los efectos del aumento de los niveles basales de glucocorticoides tanto a nivel fisiológico como a nivel de comportamiento dependen a su vez de factores tales como el tiempo durante el que se prolongue su aumento o la magnitud del propio aumento. En principio, el aumento de los niveles basales de glucocorticoides en respuesta al estrés es adaptativo y está orientado a aumentar las probabilidades de supervivencia inmediata. Sin embargo, cuando esta respuesta adaptativa y puntual al estrés se va prolongando a lo largo del tiempo y pasa a convertirse en algo crónico, aparecen los efectos dañinos del estrés que pueden incluso producir la muerte del individuo.

Dado que el estrés y la respuesta al mismo tienen efectos tanto a nivel filológico como conductual, pueden afectar a la eficacia biológica tanto directa (nivel fisiológico) como indirectamente (modulando las respuestas de comportamiento). Es por tanto fundamental investigar estos posibles efectos a la hora de tratar de entender como los animales son capaces de sobrevivir y adaptarse al medio que les rodea. Igualmente importante es tratar de entender la respuesta que se produce ante los conflictos potenciales que pueden aparecer entre las acciones encaminadas a cubrir necesidades habituales y acciones encaminadas a lidiar con el factor generador de estrés. Comprender cómo reaccionan los distintos organismos al estrés y hasta donde llega el alcance de sus efectos puede

ser fundamental a la hora de desentrañar las interacciones entre las distintas especies y la evolución de estas relaciones.

A pesar del gran número de trabajos que estudian los efectos del estrés, de la respuesta al estrés y de los propios glucocorticoides, aun quedan bastantes puntos por cubrir, sobre todo en relación a los efectos sobre decisiones conductuales aparentemente sencillas pero directamente relacionadas con la eficacia biológica individual. Esta tesis pretende ayudar a aclarar algunas de estas incógnitas usando como modelo a la lagartija de turbera (*Lacerta vivipara* Jacquin, 1787). Para ello se estudiaron, por un lado, las decisiones relacionadas con la selección de hábitat: respuestas sencillas frente a amenazas potenciales concretas en distintas situaciones con diferentes grados de riesgo. Por otro lado, se estudiaron respuestas más complejas frente a una de las decisiones más importantes para la eficacia biológica de los individuos: selección de pareja y comportamiento relacionado con la reproducción. Con todo esto se pretende llegar a entender mejor los patrones que rigen tanto la modulación como la evolución del comportamiento y de las relaciones sociales, y por tanto de las distintas estrategias vitales en esta especie como respuesta al estrés, tratando de integrar los resultados obtenidos en un contexto ecológico.

Chapter 1

1 Introduction

Stress

The concept of stress

"Everybody knows what stress is and nobody knows what it is" (Selye 1973)

Stress is a fact that is constantly around us. However, in spite of being very well known it is too little understood (Steckler *et al.* 2005). Everyone has an idea of what stress is but how to define it? In the Oxford English Dictionary "stress" is defined as "pressure or worry resulting from mental or physical distress, difficult circumstances, etc" (Horby 1989). However, stress cannot be straightforwardly defined as nervous tension (which is a common belief) given that lower animals without nervous systems and plants suffer from stress (Selye 1973). Therefore, what is the most clear biological or medical definition of stress? Few attempts to really define it have been done even when it is a term that has become part of our every day vocabulary (Selye 1973).

During the 30's Hans Selye first adopted the term "stress" (Selye 1936) for describing the patho-physiological symptoms showed by rats exposed to a series of noxious agents (Selye 1936; Berczi 1997). The symptoms showed by the rats appeared independently of the nature of the noxious agent presented and started with a "general alarm reaction" between 6-48 hours after the exposure (Selye 1936). The alarm reaction was defined by Selye (1946) as "the sum of all non-specific systemic phenomena elicited by sudden exposure to stimuli to which the organism is quantitatively or qualitatively not adapted". It is characterized by: the enlargement of the adrenal gland; the shrinkage of the thymus and other lymphoid organs and tissues; ulcerations and haemorrhages especially in the gastrointestinal tract; loss of muscular tone; fall of body temperature; and sometimes even proptosis, increased blood flow to the skin, and elevated lachrymation and salivation may appear. When

the cases are really harsh it has also been observed the apparition of liver focal necrosis and clouding of the crystalline lens (Selye 1936). Then a period of "resistance" started 48 hours after the exposure to the noxious agent in which apart form the above symptoms general body growth stopped, the gonads became atrophic; and even milk secretion stopped in lactating animals, as well (Selye 1936). This stage of resistance was defined as the "sum of all non-specific systemic reactions elicited by prolonged exposure to stimuli to which the organism has acquired adaptation as a result of a continuous exposure" (Selye 1946). The resistance developed in the second phase was sometimes so intense that affected organs returned to their original state once the noxious agent disappeared (Selye 1936). Finally, a stage of "exhaustion" turns up. "The stage of exhaustion represents the sum of all non-specific systemic reactions which ultimately develop as the result of very prolonged exposure to stimuli to which adaptation had been developed, but could no longer be maintained" (Selye 1946). However, during this third phase, the stress response ended and its associated pathology then appeared (Nelson 2005d). Therefore, after a period of 1 to 3 months if the noxious agent did not disappeared or its intensity was again enhanced then the symptoms associated with the general alarm reaction reappeared and even the death of the individual may occur (Selye 1936). Selye (1936) reported that all these symptoms seem to be an effort of the organism to adapt to the new conditions (noxious agent) so he named this process the "general adaptation syndrome" and defined it "as the sum of all non-specific, systemic reactions of the body which ensue upon long continued exposure to stress" (Selye 1946).

Nevertheless it was not until the 40's when the first comprehensive concept of the term stress was published (Selye 1946). He described the "stress response" in terms of adrenal hypertrophy, involution of the thymus, lymph nodes, spleen and other lymphoid organs and tissues. Lately, Selye (1973) reviewed the evolution of the concept "stress" and defined it as "the nonspecific response of the body to any demand made upon it" (Selye 1973). It is very important to explain the meaning of "nonspecific" in order to be able to understand this latest concept (Selye 1973). Generally, a specific agent causes a specific response. For example, the organism of a person suffering from allergy to a specific food is always going to respond exactly in the same way to that food. However, Selye (1946) stated that during the course of their work they were shocked by

the fact that a great variability of agents independently of their nature always elicited the same pathological and biochemical changes in the organism. Therefore, those agents elicit a nonspecific response in the organism. It is the sum of these non-specific adaptive reactions what Selye (1946) named the "general adaptation syndrome."

However, despite the above definitions the term stress continued under a great confusion during the 90s (Chrousos 1998). Chrousos (1998) considered that for the completely understand of what is stress, four key concepts must be clear: homeostasis, stressor, stress, and adaptive response. Homeostasis is the complex and dynamic equilibrium followed by the organism in order to maintain life, and that is in an invariable disturbance produced by intrinsic or extrinsic aversive agents or stressors (Chrousos 1998). Stress was then defined by Chrousos (1998) as a state in which homeostasis becomes threatened and thus a combination of physiologic and behavioural complex adaptive responses of the organism is produced in order to re-establish it.

On the contrary, many authors considered that the term stress should be avoided because its definition is not clear and always leads to confusion (see for example Rushen 1986). Therefore, other authors had used the term "allostasis" instead of homeostasis for defining stress (Sapolsky 1994). Allostasis is the process by which the organism achieves stability (homeostasis) through physiological or behavioural adjustments (Nelson 2005d). However, during the acquisition of the stability the organism incur in a series of cumulative costs that have been defined as "allostatic load" (McEwen and Wingfield 2003; Nelson 2005d). An animal might not have problems to cope with high allostatic loads if its general state is good and the environmental conditions that is supporting are good as well. However, under bad conditions the energy required to cope with a high allostatic load might exceed individual's capacity resulting in an "allostatic overload" (McEwen and Wingfield 2003; Nelson 2005d). Long lasting stressors lead to an allostatic overload (Nelson 2005d) and allostatic overload might lead to serious pathological and physiological consequences (McEwen and Wingfield 2003). McEwen and Wingfield (2003) proposed two types of allostatic overloads and thus two types of potential stress responses. Type 1 allostatic overload occurs when the energy demand surpasses the energy supply. This activates an "emergency life history stage" in which the animal enters into a

survival mode in order to reduce the allostatic load and to recover a positive energy balance so normal life cycle may be achieved again (McEwen and Wingfield 2003). In contrast to, when enough energy consumption exists so energy demands is not exceeded but social conflict appears becoming the primary force driving to allostatic load, then type 2 allostatic overload begins. Therefore, type 2 allostatic overload can only be counteracted by learning and by social changes (McEwen and Wingfield 2003). Type 2 allostatic overload is the typical case in animals living in captivity and thus with all their basic requirements covered but with an altered social structure (McEwen and Wingfield 2003).

Recently Kim and Diamond (2002) suggested a three-part definition of the concept of stress that might be more easily understandable and that unifies the interaction among stress, hormones implied and behaviour (Nelson 2005d). This new definition is valid for all species including humans as well. First, stress elicits an increased state of arousal that can be determined by the measure of brain electrical activity, behaviour such as locomotor activity or by some specific hormonal levels (see below *Stress response and physiology of stress*; *Neuroendocrinal mechanisms and hormones implied*; see also Kim and Diamond 2002). However, that state of arousal can be achieved under both pleasing and aversive situations. So, second, for an event or situation to be defined as stressful the individual must have a negative or aversive perception over it. Kim and Diamond's (2002) stated that the level of aversiveness of a given stimulus can be measure depending on how acutely an individual tries to avoid or to diminish the intensity of the stressor they are facing if given the opportunity. The third component of Kim and Diamond's (2002) definition of stress is "controllability" given that the degree of stressfulness of a given experience is greatly attenuate by the control that the individual has over the aversive situation. Therefore, the element of control and the related concept of "predictability", that may be developed by the previous experience of the same aversive situation, are the variables that at last determines the extent of the stress experience. The susceptibility of the individual to develop long-term negative effects derived from the stressful situation is also defined by its controllability of the aversive situation. Consequently, stress is then considered by Kim and Diamond (2002) to be a condition in which individuals are aroused by a perceived as aversive situation, being the extent of the stress and thus its physiological implications determined by the

individual's perception of its ability to control over the aversive situation.

Stress response and physiology of stress

Neuroendocrinal mechanisms implied

When a stressor disrupts homeostasis the individual elicits the stress response. The stress response is thus the collection of physiological and behavioural responses that leads to the restoration of homeostasis (Nelson 2005d). As mentioned before, the stress response is nonspecific so very different stressors elicit a similar physiological response (Selye and Fortier 1950; Selye 1973).

The hypothalamic-pituitary-adrenal axis (HPA-axis) is involved in the stress response by the regulation of a complex suite of different hormones that are released once a stressor disrupts homeostasis (Box 1. 1.; Figure 1. 1.). Initially, the disruption of homeostasis causes an immediate release of catecholamines: adrenaline, from the adrenal medulla, and noradrenaline, from the sympathetic nervous system. Both catecholamines stimulate the respiratory and cardiovascular systems by increasing respiration and heart rates, and blood pressure. Both adrenaline and noradrenaline act also by increasing blood glucose levels. However, adrenaline acts in the first place because a lower increase in their basal levels than in noradrenaline is needed in order to elicit its effects. After the release of catecholamines the hypothalamus releases corticotropin-releasing hormone (CRH) that stimulates the anterior pituitary gland in order to produce adrenocorticotropic hormone (ACTH), β-endorphin and prolactin (Figure 1. 1.; Buckingham *et al.*. 1997; Randall *et al.* 2004; Nelson 2005d). The β-endorphin is one of the endogenous opioids coming from the cleavage of the larger protein pro-opiomelanocortin (POMC). POMC is the precursor for ACTH as well. Stressful conditions trigger the release of β-endorphin in order to reduce pain perception (Nelson 2005a). However, each kind of stress elicit a different prolactin-secretory response and thus it is not possible to describe a general mechanism but each specific mechanism associated with each specific kind of stress needs to be described (Freeman *et al.* 2000; Angelier and Chastel 2009). During the stress response the posterior pituitary gland releases vasopressin as well. Vasopressin acts in order to increase blood volume and pressure for making the delivery of energy to muscles more efficient (Nelson 2005d). Finally, ACTH stimulates de adrenal cortex so it segregated glucocorticoids (see Box 1. 1. and Figure 1. 1.). Therefore, the result of the disruption of homeostasis is the final release of glucocorticoids few minutes after the stressor acted.

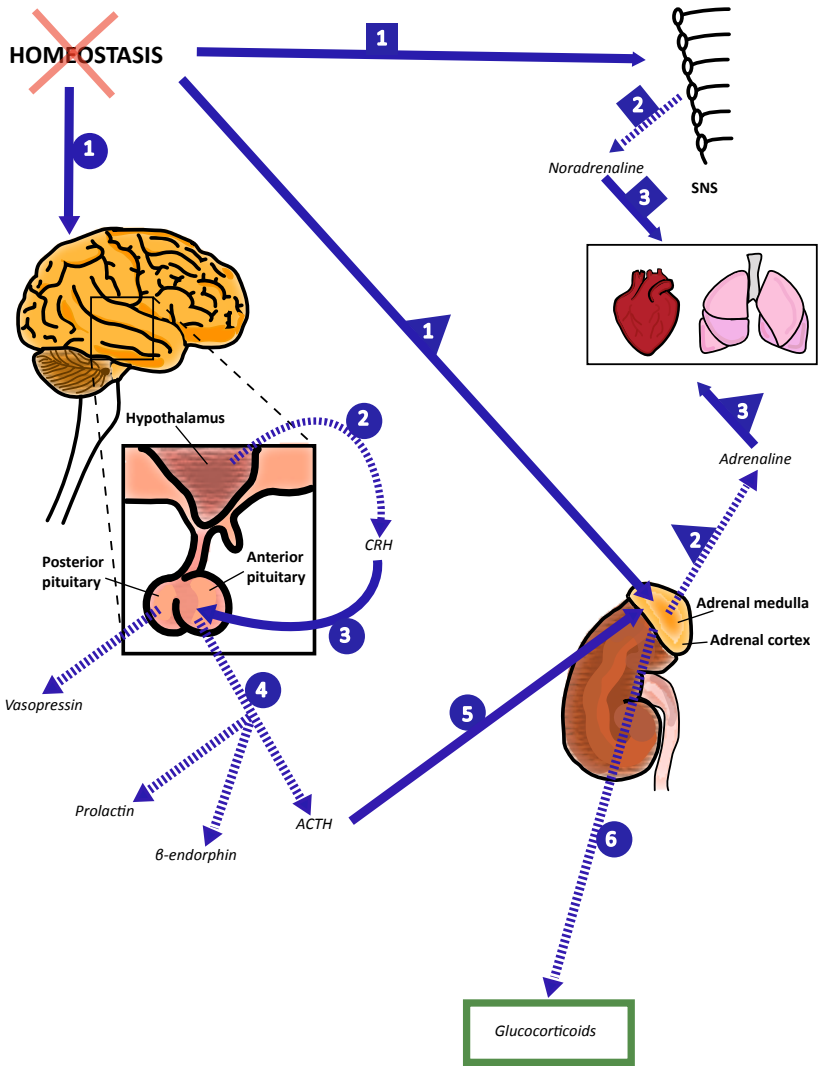

Figure 1. 1. Physiological response to the disruption of homeostasis. When a stressor causes the disruption of homeostasis adrenaline is secreted from the adrenal medulla, and noradrenaline from the sympathetic nervous system (SNS) in order to increase respiration and heart rates, blood pressure, and blood glucose levels. Then the hypothalamus releases corticotropin-releasing hormone (CRH) that stimulates the anterior pituitary gland to produce adrenocorticotropic hormone (ACTH), β-endorphin and prolactin. The posterior pituitary gland releases vasopressin during the stress response. Finally, ACTH stimulates de adrenal cortex so it segregates glucocorticoids. Blue continuous arrows indicate direct effect and blue discontinuous arrows indicate secretion. Arrows belonging to the same pathway are marked with the same geometrical figure (squares for noradrenaline production and effects, triangles for adrenaline production and effects, and circles for the specific HPA axis response) and with numbers following chronological order. Hormones are written in italics and locations in bold.

Glucocorticoids

The glucocorticoids are a type of corticoid (steroid hormone derived from cholesterol, Figure 1. 2.) involved in carbohydrate metabolism and often released in response to a stressful event. The other type of corticoids are the mineralocorticoids, the most important of them is aldosterone that is produced in all terrestrial vertebrates in order to regulate ion exchange and water metabolism (Nelson 2005a). Corticosterone and cortisol are the two primary glucocorticoids. Most animals produce both corticosterone and cortisol but only one of them in large quantities. Corticosterone is the primary glucocorticoid secreted by reptiles, amphibians, birds, rodents and other small mammals such as lagomorphs, while cortisol is secreted in fish and the other mammals (Figure 1. 2.; Nelson 2005a).

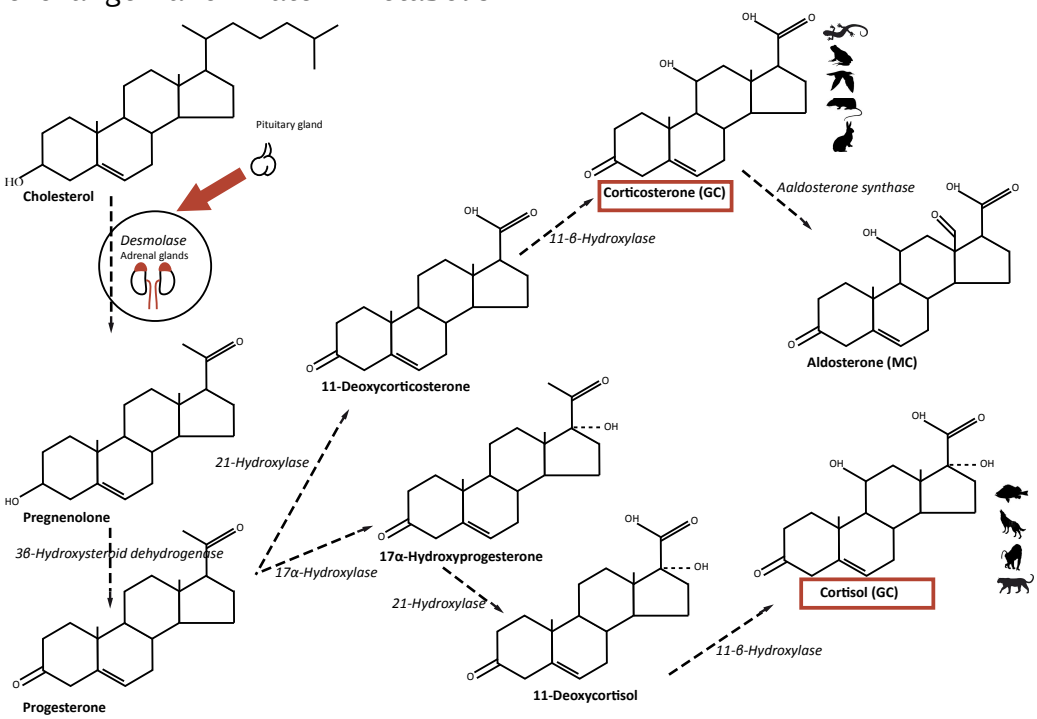

Figure 1. 2. Corticoids' simplified biochemical pathway formation. Cholesterol is converted into steroid hormones in the adrenals by the action of desmolase, a pituitary hormone that cleaves off the long chain of carbons from the top of the cholesterol molecule, in order to produce pregnenolone. Pregnenolone is a prohormone that it is the obligatory precursor of corticoids. There are two types of corticoids: glucocorticoids (GC) and mineralocorticoids (MC). Corticosterone (GC) is the glucocorticoid secreted by reptiles, amphibians, birds, rodents and other small mammals and it is also the precursor of aldosterone (MC) that regulates ion exchange and water metabolism. Cortisol (GC) is the glucocorticoid secreted in fish and the other mammals. The enzymes that are involved in the production of the hormones are shown in italics (Nelson 2005a).

Glucocorticoids act in the liver by increasing the gluconeogenesis related enzymes production (Sapolsky *et al.* 2000; Randall *et al.* 2004). The gluconeogenesis produces glucose formation from non-carbohydrate molecules that might be converted to glycogen and stored in the liver and muscles. However, most of the new synthesized glucose is released into the circulating blood eliciting an immediate increase of blood glucose levels. In addition, glucocorticoids also reduce glucose delivery to peripheral tissues such as muscles and therefore they indirectly increase glucose blood levels by this way as well. Simultaneously, aminoacids delivery to muscular tissues is also inhibited by glucocorticoids secretion. Therefore, blood aminoacid levels increase as well and the liver is able to use those extra aminoacids for deamination and for their conversion into glucose when stimulated by glucocorticoids. Consequently, when glucocorticoids levels increase they induced an increase in blood glucose levels by two pathways: an increase in the gluconeogenesis produced in the liver, and by the arrival of extra aminoacid to the liver that are used as gluconeogenesis precursors (Figure 1. 3.). This process is quite important during fasting when a great degradation of the proteins from different tissues is produced in order to maintain blood glucose levels and thus, a good energetic supply in critic tissues such as brain. Deamination produced by glucocorticoids in the liver helps to get rid off the free amino groups derived from the protein degradation during fasting. Glucocorticoids also stimulate the mobilization of fatty acids from the adipose tissue. Fatty acids might be used as gluconeogenesis precursors in the liver, or might be directly metabolized in the muscles in order to produce energy for muscle contraction (Figure 1. 3.). Therefore, glucocorticoids act on different metabolic pathways in order to replenish the energy reserves and most importantly in order to enhance the immediate energy availability in muscle and nervous tissues (Randall D *et al.* 2004). Glucocorticoids have many other functions that will be mentioned later, such as the stimulation of gastric secretions and the inhibition of the immune response (Sapolsky *et al.* 2000; Randall *et al.* 2004).

Basal glucocorticoids levels are regulated as mentioned before by the production of CRH and ACTH. However, glucocorticoids are also regulated by a strong circadian rhythm resulting from the daily variation in CRH levels (Nelson 2005b). Cortisol in humans has the highest basal level around awakening (beginning of daily activity) and its lowest value during midnight (Mills 1966; Besser and

Box 1. 1. Hormones implied in the stress response. Shown are the primary hormones implied in the stress response with their structure, principal tissues of origin, goal tissues and function (Vamvakopoulos and Chrousos 1993; Raadsheer *et al.* 1994; Buckingham *et al.* 1997; Freeman *et al.* 2000; Randall *et al.* 2004; Nelson 2005a; Angelier and Chastel 2009).

Hormone	Structure	Tissue of origin	Goal tissue	Principal function
Adrenaline, noradrenaline	Catecholamine	Adrenal medulla	Almost all tissues	Increases respiration and heart rate. Induces vasoconstriction. Induces glycolysis, hyperglycemia and biolysis.
β-Endorphin	Neuropeptid	Anterior pituitary gland	CNS, PNS *	Stops pain perception and inflammatory response.
CRH	Peptide	Paraventricular nucleus (hypothalamus), T-lymphocytes, placenta	Pituitary	Stimulation of the pituitary synthesis of ACTH, β-endorphin and prolactin. Marker that determines the length of gestation. Proinflammatory action.
ACTH	Polypeptide	Anterior pituitary gland	Adrenal cortex	Increased production and release of glucocorticosteroids.
Prolactin	Polypeptide	Anterior pituitary gland (lactotrophs), brain (hypothalamus and other parts), uterus, placenta, breast, lymphocytes, prostate	A lot of them	Important regulator of the immune system, osmoregulation, hematopoiesis, angiogenesis. Promotes lactation, maintains and promotes secretory activity of the corpus luteum, and other actions related to reproduction (mating and maternal behaviours).
Vasopressin	Nonapeptide	Neurohypophysis	Kidney	Maintenance of osmotic balance and blood pressure regulation
GC**	Steroid	Adrenal cortex	Liver, adipose tissue	Enhances amino acids mobilization in the muscles and gluconeogenesis in the liver in order to increase glucose blood levels. Enhances the level of fat acids in the liver coming from adipose tissue. Anti-inflammatory function.

* CNS = Central Nervous System, PNS = Peripheral Nervous System; **GC = Glucocorticoids

Butler 1967). In birds and reptiles, this peak in glucocorticoids levels (corticosterone) has also been found before their period of maximum activity (Chan and Callard 1972; Meier and Ferrell 1978; Woodley *et al.* 2003). However, corticosterone cycle is reversed in rodents and other nocturnal animals and thus, the highest basal levels are found at the beginning of the night activity period (Guillemin *et al.* 1959; Thanos *et al.* 2009). In addition, in animals with biphasic activity like alligators there are two peaks in corticosterone levels just prior to their activity periods (Lance and Lauren 1984).

There are also seasonal variations in glucocorticoids levels. Romero (2002) published a review showing that the level of glucocorticoids in free-living reptiles (Grassman and Hess 1992; Jessop *et al.* 2004; Phillips and Klukowski 2008; Lutterschmidt *et al.* 2009), amphibians (Pancak and Taylor 1983; Denari and Ceballos 2005, 2006), and birds (Wingfield *et al.* 1994; Romero *et al.* 1997; Romero *et al.* 1998; Romero and Wingfield 1998; Nelson 2005d) were higher during the breeding season when energy demands are increased. This was not the case in mammals (Kenagy and Place 2000; Nunes *et al.* 2006; Schradin 2008), The seasonal changes in glucocorticoids levels are thus very different depending on each species and even in very close species, making the task of describing a general pattern very difficult. For example, in some reptile species no seasonal variation has been found (Amey and Whittier 2000; Ott *et al.* 2000; MacDonald *et al.* 2007). The same has been found for some amphibians (Homan *et al.* 2003). In addition, it has been described an increase in glucocorticoids levels in some mammals during breeding season (Romero *et al.* 2008) but also during winter when conditions are most demanding (Amirat *et al.* 1980).

Stress response and physiological effects

Summarizing what it was previously written, immediately after exposure to a stressor, a physiological stress response begins. The sympathetic nervous system secretes noradrenalin and the adrenal medulla secretes adrenalin. The activity of the HPA-axis is then stimulated with a final result of an increase of the glucocorticoids levels within few minutes of the perception of the stressor. The stress response is believed to have many adaptive effects (Nelson 2005d). Firstly, *(a)* an immediate increase in the availability of energy is produced few seconds after the stressor perception due to

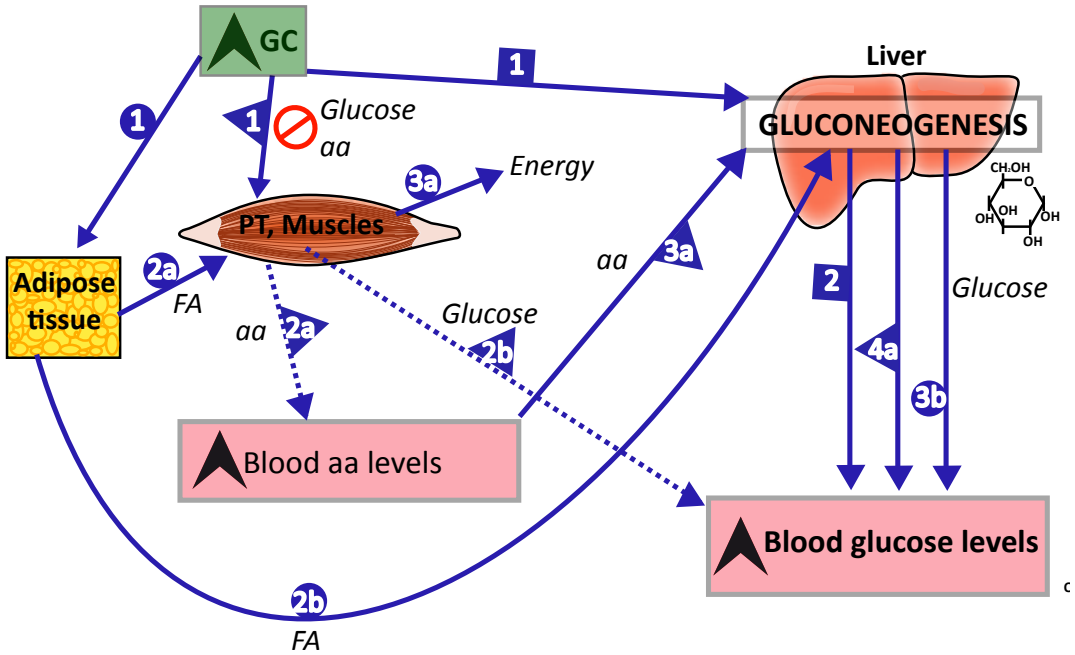

Figure 1. 3. Glucocorticoids (GCs) related pathways producing increase in blood glucose levels. GCs enhance gluconeogenesis in the liver in order to produce an immediate increase of blood glucose levels (pathway marked with squares). GCs reduce glucose delivery to peripheral tissues (PT) and thus indirectly (discontinuous arrow) increase blood glucose levels, while aminoacids delivery to muscles is also inhibited producing an increase in blood aminoacid (aa) levels that might be used for gluconeogenesis as well (pathway marked with triangles). GCs also mobilize fatty acids (FA) from the adipose tissue that might be used as gluconeogenesis precursors or might be used in the muscles for producing energy for contraction (pathway marked with circles). Arrows belonging to the same pathway are marked with the same geometrical figure and with numbers following chronological order. Molecules and metabolic products are written in italics and locations in bold.

the action of catecholamines that increase blood glucose levels. Secondly, the release of glucocorticoids increases gluconeogenesis few minutes later (Wingfield *et al.* 1998; Randall *et al.* 2004). In addition, stress response produces *(b)* an increase in the oxygen intake due again to catecholamines that increase respiration and cardiovascular rates (Sapolsky *et al.* 2000; Randall *et al.* 2004; DuRant *et al.* 2008). In addition, *(c)* adrenalin increases blood flow to areas necessary for movement (muscles) and a decrease to energetically expensive areas and processes not necessary for movement and thus, not critical for coping with the stressful event (digestion, growth, immune function and reproduction). Vasopressin also increases blood volume and pressure for making the delivery of energy to the muscles more efficient, and prolactin decreases

reproduction temporarily (Buckingham *et al.* 1997). *(d)* β-endorphin produced during stress response decreases pain perception. Finally, *(e)* stress response produces an enhancement of senses and memory (Shors *et al.* 1992; Abercrombie *et al.* 2003) due to the existence of glucocorticoids receptors in the hippocampus that are activated just under high glucocorticoids elevations (Reul and Kloet 1985; Roozendaal 2003), and by the action of vasopressin that enhance memory consolidation. Therefore, acute stress response improves survival in the first place. However, if this acute stress response is not ended or if it is initiated too often the pathological related consequences arise (Nelson 2005d).

Therefore, chronic stress turns adaptive acute stress response into a pathological condition that may seriously compromise health and survival (Nelson 2005d) due to the prolongation of the above mentioned physiological stress response effects. Selye (1946) named as "diseases of adaptation" all those pathological conditions that were not directly due to any particular pathogen but to the stress response itself. A constant energy mobilization with no energy storage implies that the organism would never have any extra energy to use in a long-term period, and thus it will fatigue more rapidly, and the risk of developing a form of diabetes will even increase (Sapolsky 1994). A constant increase in cardiovascular tone, sympatho-adrenal activation and long-term glucocorticoids elevations lead to hypertension and atherosclerotic plaques that may contribute to produce heart diseases, embolisms and strokes (Sapolsky 1994; Kaplan and Manuck 1999; Li *et al.* 2004; Nelson 2005d). In addition, myopathy may be also derived from prolonged corticosterone elevations (Sapolsky 1994; Nelson 2005d). In addition, the inhibition of digestion during stress response may alter appetite and even lead to gastrointestinal disease and peptic ulcers (Selye 1936; Sapolsky 1994).

Obviously, one the most detrimental chronic stress effects is the impairment of immune system (Sapolsky 1994). Glucocorticoid elevations typically produce an inhibition of the inflammatory response and of the antibody production (Mader 2006). During stress response there is an inhibition in the formation of new lymphocytes and their release into the circulation, and a disruption of the communication among lymphocytes through the release of relevant messengers (Isobe and Lillehoj 1993; Sapolsky 1994; McEwen *et al.* 1997; McEwen 2000; Berger *et al.* 2005). Glucocorticoids elevations also produced thymus,

spleen, lymph glands and liver involution (Selye 1936). All this may lead to impaired disease resistance or even cancer (Ben-Eliyahu *et al.* 1991; Nelson 2005d).

As mentioned before, during stress response reproductive function is inhibited. This is due primarily to the action of prolactin that is secreted from the anterior pituitary gland and acts in the hypothalamic-pituitary-gonadal axis (HPG axis) at multiple sites (Nelson 2005d). Prolactin at high concentrations impairs testosterone production.
Furthermore, while β-endorphin inhibits pain perception, it also suppresses gonadotropin-releasing hormone (GnRH). GnRH regulates the production in the anterior pituitary of follicle-stimulating hormone (FSH, stimulates the development of follicles in females and the production of sperm in males) and luteinizing hormone (LH, enhances the development of the corpora lutea in females and the production of testosterone in males; Nelson 2005d). Thereby, the inhibition of GnRH impairs reproduction. In addition, glucocorticoids may also affect reproduction given that they directly modify the circulating blood levels of some sex steroids and other related to reproduction hormones (Cunningham *et al.* 1975; Moore and Zoeller 1985; Tokarz 1987; Brann and Mahesh 1991; DeNardo and Licht 1993). In addition, in many species stress negatively affect to both males and females proceptivity and receptivity behaviours during reproduction (Wingfield and Sapolsky 2003)

Finally, even when an acute stress response facilitate memory enhancement, long-term glucocorticoids elevations have detrimental effects on learning and memory (Luine 1994; Luine *et al.* 1994; McEwen and Sapolsky 1995; Lupien and McEwen 1997; de Quervain *et al.* 1998; Kim and Diamond 2002). The hippocampus plays an important role as centre of learning and memory for birds and mammals (Day *et al.* 2001). Hippocampal neurogenesis in rodents and primates is mediated by blood glucocorticoids concentration with higher concentrations suppressing the production of hippocampal neurons (Abercrombie *et al.* 2003). In addition, glucocorticoids produce neuronal death (Sapolsky 1996) and alter hippocampal dendritic morphology (Sapolsky 1996; Gould *et al.* 1998; Kim and Diamond 2002). Therefore, memory and learning are impaired partly by the chronic suppression of hippocampal neurogenesis caused by chronic stress and by its associated elevation of glucocorticoids blood levels (Kim and Diamond 2002; Nelson 2005c). The effect of stress on learning and memory depends

on its duration and also on the magnitude of the increase of its related glucocorticoids. On one hand, long lasting stressors produce chronic stress that decreases memory as a long-term treatment with glucocorticoids (de Quervain *et al.* 1998; McLay *et al.* 1998). On the other hand, a punctual stressor produces an acute stress response that promotes long lasting memories and enhances learning and memory as occurs with a punctual application of a glucocorticoids' treatment (Shors *et al.* 1992; Buchanan and Lovallo 2001). Furthermore, long-term moderate elevation of glucocorticoids appeared to enhance spatial memory (Pravosudov 2003). Positive effects of glucocorticoids on learning and memory seem to be related with the amygdala that modulates memory consolidation processes (Nelson 2005c).

Effects of Stress on Animal Behaviour

The effects of stress: Behavioural stress response

The stress response not only change physiology in order to cope with the stressor, it also affects individuals' behavioural response in order to promote immediate survival. The first component of the physiological stress response produce an increase in respiratory and heart rates, and an increase in blood flow to the muscles (catecholamines). The animal thus enters in the emergency life history stage and its normal behaviour changes for optimizing survival probabilities (McEwen and Wingfield 2003). During this stage there is an increase in the state of vigilance especially when the threat would not be clearly avoid using any alternative behavioural response, foraging increases and there is irruptive-type migration during the day (Wingfield and Kitaysky 2002; McEwen and Wingfield 2003). All this prepares individuals so they are able to perform a "fight or flight-response" (see Quick and Spielberger 1994) that it is the first component of the behavioural stress response. This first component includes for example, the development of antipredator defensive behaviours (*e.g.* López and Martín 2001; Martin and Lopez 2001), or the activation of the physical mechanisms needed. For example, in reptiles, it has been shown how they behaviourally increase their body temperature under stressful conditions (Cabanac and Bernieri 2000). The raising of body temperature in reptiles means an increase in physiological performance in terms of speed and stamina (Moberly 1968; John-Alder and Bennett 1981; Bennett 1983; Chen *et al.* 2003; Pinch and

Claussen 2003) what may result in a more efficient physical response.

If the stressful event persists then another component of the behavioural stress response follows the first fight or flight-response. This second component includes a coping strategy named as "take-it-or-leave-it strategy" that includes several sub-strategies (Wingfield and Kitaysky 2002; Wingfield 2003): *(a)* "Leave-it strategy", the animal simply moves away from the stressor itself; *(b)* "Take-it strategy", the stressor persists so the animal change its behaviour in order to enhance energy saving (*e.g.* it looks for a refugee); and *(c)* "Take at first and then leave-it strategy", the animal first tries to save energy but if stressor persists then moves away from it (Wingfield and Kitaysky 2002; Wingfield 2003).

The threat-sensitivity hypothesis predicts that individuals may act depending on the magnitude of the threat they are facing (Helfman 1989). In addition, as we have seen before the extent of the stress effects is determined by the individual's perception of its ability to control the stressful event. Consequently, behavioural stress response may be very variable depending on the species, or even depending on each individual perception, but what it is clear is that it is always oriented to enhancement of survival.

Effects of the stress response

The brain must be affected in order to promote a change in the behaviour. Therefore, because of its capacity of crossing the blood-brain barrier, and because there are many glucocorticoids receptors in several brain regions, the glucocorticoids are the primary mediators in the behavioural stress response (Nelson 2005d). In order to enhance immediate survival, glucocorticosteroids increase appetite and locomotor activity (Belliure and Clobert 2004; Cote *et al.* 2006; Miles *et al.* 2007) and thus favours food-seeking behaviour in order to promote energy intake (McEwen and Wingfield 2003; Angelier *et al.* 2007). In some cases, basking and thermoregulatory behaviour may be inhibited (Belliure and Clobert 2004). However, there are many other behaviours not directly related to promote immediate survival that are influenced by the action of glucocorticoids as well. In many cases the mechanism that influence those behaviours is not very clear (Whittow 2000). For example, volumes of research investigate glucocorticoids effects on social behaviour. On one hand, it has been shown that glucocorticoids tend to inhibit aggressive behaviour (Tokarz 1987; DeNardo and Licht 1993; Hanley and Stamps 2002). In addition, exogenous glucocorticoids may also

reduced home-range size (DeNardo and Sinervo 1994a, 1994b), and territorial behaviour (Wingfield and Silverin 1986). Even population crashes produced in some populations might be associated with high corticosterone levels (Christian 1961; Thiessen and Rodgers 1961).

On the other hand, one of the most important glucocorticoid effects is its influence on reproductive behaviour since, as mentioned above, glucocorticoids directly modify the blood levels of reproductive hormones such as testosterone. On one hand, if males aggressive behaviour is inhibited then access to females, in species showing male intrasexual competition, may be limited. The same might happen if territoriality and home-range size is reduced in species in which maintenance of a breeding territory is necessary for mating. Therefore, some of the effects of glucocorticoids on behaviour may indirectly impair reproduction success. On the other hand, high glucocorticoids levels may directly impair reproduction success by inhibiting courtship behaviour (Moore and Miller 1984; Dunlap and Schall 1995), and even parental care and effort (Silverin 1986; Angelier and Chastel 2009). However, the effect of glucocorticoids on reproduction may be very variable depending on the reproductive strategy used and sometimes there are even compensatory mechanisms that inhibit deleterious glucocorticoids effects on reproduction (see DeNardo and Licht 1993; Wingfield and Sapolsky 2003).

Another interesting effect of glucocorticoids on behaviour is its influence on dispersal behaviour. In birds, glucocorticoids seem to mediate in the initiation of dispersal activity (Belthoff and Dufty AM 1998). In addition, prenatal exposure to corticosterone may affect juveniles' dispersal depending on their body condition (Meylan *et al.* 2002) and also depending on the timing and duration of the exposure (Vercken *et al.* 2007). Other effects of prenatal exposure to stress or glucocorticoids have been found as well. For example, rhesus monkeys whose mothers experiment prenatal stress showed attention deficits, impaired cognition and social behaviours (Schneider 1992; Clarke and Schneider 1993; Clarke *et al.* 1994).

Effects of Stress on Sexual Selection

General

Sexual selection is the selection of traits that enhance mating success (Krebs and Davies 1993). The sexual selection may arise, among

other things, because of the existence of some kind of sexual conflict (see Chapman *et al.* 2003). First, females put more resources in the production of each gamete and thus, in the production of their offspring. Therefore, females increase their reproductive success by allocating more resource per gamete production than males, but males do so by fertilizing as many gametes as they can. In addition, males are able to fertilize females gamete faster than they are produced, what makes females a scarce resource for maximizing male reproduction success. Furthermore, the existence of a sex ratio 1:1 also enhances sexual selection by increasing intrasexual competition (Krebs and Davies 1993).

Sexual selection includes two components that usually act simultaneously. The first component is the intrasexual selection that indirectly favours individuals' ability to compete by for example, developing secondary sexual characters such as fighting weapons (Lappin and Husak 2005; Malo *et al.* 2005; Haley and Gray 2012; Munoz and Zink 2012). However, there are less obvious ways by which males compete with each other like sperm competition. Males try to eliminate from female genitalia previous males' sperm or avoid the entry of sperm from other males (Harshman and Prout 1994; Arnaud *et al.* 2001; Munroe and Koprowski 2012), and even try to guard females (Archer and Elgar 1999; Zamudio and Sinervo 2000; Chuang-Dobbs *et al.* 2001). It has even been found that males may deposit anti-aphrodisiac scent in females after copulating in order to prevent new male approaches (Andersson *et al.* 2004). Intersexual selection is the second component and favours traits oriented to attract the other sex as for example, brighter colorations or highly ornamented traits (Andersson 1982; Hamilton and Sullivan 2005). Because female is the sex with higher investment in gamete production, they need to be sure (whenever they are able to) that the male they are choosing is a really good quality male. Consequently, females are generally very choosy and might select males on the basis of the material resources they might supply (*e.g.* high quality territory for raising their offspring, Lenington 1980; Alatalo *et al.* 1986; high quality nests, Hoi *et al.* 1994; high quality food provisioning, Bussiere *et al.* 2005), and also for the potential genetic benefits to their offspring (*i.e.* genes that maximizes their offspring probability of surviving, competing and reproducing, Krebs and Davies 1993; Kokko *et al.* 2003). It is important to mention that females also develop secondary sexual characters in order to cope with female

intrasexual competition for a chance to breed, and for attracting males (Clutton-Brock 2009). However, in contrast to males, females often compete more intensely for accessing resources needed for a successful reproduction than for the access to males (Clutton-Brock 2009).

The intensity of sexual selection is marked by the grade of competition for potential mates (Krebs and Davies 1993) what depends on two factors: differences in the parental effort developed by each sex (Clutton-Brock 1991; Clutton-Brock and Vincent 1991; Magrath and Komdeu 2003), and the operational sex ratio (Baylis 1981; Clutton-Brock and Vincent 1991). In species where the parental effort is similar between sexes then the intensity of sexual selection is lower than in species in which one of the sexes incurs in a greater parental effort. In the latest, the sex whose parental effort is lower is the one having a higher potential rate of reproduction and thus, it is usually the one that actively compete for the access to the other sex (Clutton-Brock and Vincent 1991; Simmons 1992). The operational sex ratio is the ratio of males to females that are available for mating in a population at a given time (Emlen and Oring 1977). Therefore, operational sex ratio predicts the degree of mate competition (Kvarnemo and Ahnesjo 1996; Kokko and Monaghan 2001). For example, in a population when both sexes enters in breeding condition at the same time, then one male may only be able to control or defend a group of just few females, and that male may be defenceless against other males specially during copulating. However, if the females enter asynchronously in breeding condition, there would be less females available at the same time an thus, the same males would be able to control a larger group of females at the end (one after the other). Therefore, male intrasexual competition is harder in the latest case (Krebs and Davies 1993). However, recent studies suggest that the intensity of sexual selection not only depends on parental investment and operational sex ratio. The different components of each mating system, such as other cost of breeding apart of parental investment (Kokko and Monaghan 2001; Fitze and Le Galliard 2008), or the use of alternative breeding strategies (Mills and Reynolds 2003), may also play an important role in determining the intensity of sexual selection.

Effects of stress and stress response on sexual selection

Apart from glucocorticoids direct effect in reproductive behaviour as previously mentioned, there is a

clear variation in glucocorticoids levels depending on the season (Romero 2002) but even depending on sexes (Grassman and Hess 1992; Goymann and Wingfield 2004) and social status (Gust *et al.* 1993; Creel 2001; Goymann and Wingfield 2004). This fact makes glucocorticoids a key element for the direct action of sexual selection (Husak and Moore 2008). Individuals may show preferences both for mates with the lowest basal glucocorticoids levels or with the highest glucocorticoids levels (Husak and Moore 2008). These may be due to the fact that glucocorticoids may affect many condition-dependent sexually selected traits (*i.e.* honest signals, Kodricbrown and Brown 1984; Husak and Moore 2008).

On one hand, if mating occurs just for direct benefits then sexual selection may benefit lower glucocorticoids levels because high levels are negatively related to the achievement of those direct benefits. For example, territorial behaviour is inhibited by high glucocorticoid levels in male song sparrows (*Melospiza melodia*, Wingfield and Silverin 1986), and body mass of nestlings is negatively correlated with their parents' glucocorticoids levels in barn swallows (*Hirundo rustica*, Jenni-Eiermann *et al.* 2008). Consequently, direct benefits coming from mating those males might be lower than direct benefits coming from males with lower glucocorticoids levels.

On the other hand, mate choice also occurs for indirect benefits so offspring produced from better quality males would be in advantage when trying to survive, compete or reproduce. Since glucocorticoids levels are heritable (see Roberts *et al.* 2007a; Roberts *et al.* 2007b), then when looking for good quality mates, choosing one with higher glucocorticoids levels may result in bad quality offspring because of the deleterious chronic effects of high glucocorticoids elevations. First, high glucocorticoids levels may negatively affect one of the most important traits for mate choice: body condition (Sockman and Schwabl 2001; Berger *et al.* 2005). In addition, high glucocorticoids levels impair immune system (see Oppliger *et al.* 1998; Wingfield and Kitaysky 2002; Berger *et al.* 2005). Therefore, selecting mates with increased glucocorticoids levels may result in offspring having poor survival prospects because of their poor body condition or simply because they would be more susceptible to diseases or parasites. Second, for optimizing offspring ability to compete with other conspecifics, mates with low glucocorticoids levels might be selected as well. On one side, the inhibition of aggressive behaviour produce by high glucocorticoids

levels (Nock and Leshner 1976; Roche and Leshner 1979; DeNardo and Licht 1993) may impaired offspring competing ability. On the other side, if home-range size is reduced by high glucocorticoids levels, as in male side-blotched lizards (*Uta stansburiana,* DeNardo and Sinervo 1994a), then those males would have reduce competing and reproductive success, because they can access less females than males with bigger home-ranges sizes. Therefore, their offspring may have less competing capacities than the offspring coming from males with lower glucocorticoids levels. Finally, high glucocorticoids levels are negatively related to other traits that might increase mate attractiveness, such as birds' song quality (Spencer *et al.* 2003; Buchanan *et al.* 2004; Spencer *et al.* 2005; MacDougall-Shackleton *et al.* 2009). In amphibians, it has also been negatively related to the quality of males' vocalizations (Leary *et al.* 2006a; 2006b) and courtship behaviour (Moore and Miller 1984). Some studies, also link high glucocorticoids levels with visual cues used for sexual selection as plumage quality (DesRochers *et al.* 2009) and colouration (Roulin *et al.* 2008). Consequently, selecting mates with lower glucocorticoids levels may increase offspring attractiveness and thus future mating probability. In fact, it is known that stress response might be downregulated during periods in which sexually selected traits may be affected (see Romero 2002), *e.g.* during courtship (Wingfield and Sapolsky 2003) what signals its great influence in sexual selection (Husak and Moore 2008).

Something of concern, when trying to investigate the effect of stress and glucocorticoids on sexual selection is that there is a two-way interaction between glucocorticoids, sex steroids and body condition that may influence the quality of sexually selected traits (Husak and Moore 2008). Therefore, it is possible that sexual selection does not directly act on glucocorticoids levels but on the traits that they may affect (Husak and Moore 2008) and differing between both is a very complex task. In addition, even when glucocorticoids suppress reproductive activity they may enhance energetically expensive reproductive behaviour by its role in energy mobilization (Romero 2002; Moore and Jessop 2003). Therefore, glucocorticoids may play an important role in sexual selection. However, their exact role and the mechanism under which they act are still under debate.

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Chapter 2

2 Goals

The general objective of this thesis is to provide new insights into the implications of stress and the physiological stress response for animal behaviour. More precisely and taking into account that I use as model species the common lizard (*Lacerta vivipara* Jacquin, 1787), whose physiological stress response induces an increase in the basal corticosterone levels, we are interested in the corticosterone effects on common lizard behavioural responses. It is known how the physiological stress response is produced in this species but despite the amount of studies that investigate common lizard behaviour in response to stress, to my knowledge there are no studies showing the effect of stress or corticosterone on some important fitness related behaviours such as microhabitat selection in response to natural stressors (*i.e.* potential threats) or on mate choice, reproduction related behaviours or on intersexual social interactions.

As introduced above, stress response may lead animals to behavioural changes in order to achieve stability again. Therefore, studying behavioural decisions potentially affecting animal fitness under natural stressors may lead us to a better understanding of the behavioural stress response and its implications on ecology and evolution. In addition, investigating how animals with increased corticosterone levels behave in a given situation may help us to understand the effect of the physiological stress response at the behavioural level. Mating season is one of the most stressful moments in animals' life. Consequently, investigating how stress response affects mating behaviour and mate choice may help us to achieve a better understanding of forces driving sexual selection. Finally, given that both duration and magnitude of the corticosterone elevation determine the physiological consequences of stress and the stress response, investigating dose-dependent effects

of corticosterone may be also determinant to fully understand the behavioural stress response. Consequently, in this PhD thesis I investigated the following questions:

- 1 (Chapter 4). *Does stress affect lizard's microhabitat selection?* In order to test the outcome of behavioural stress response under natural stressors, I experimentally investigated microhabitat selection of the common lizard, a crucial parameter for lizards' fitness. I ran an experiment that allowed us to understand if microhabitat selection is the result of multifactorial integration based on series of factors that might be a potential source of stress for lizards in their natural environment: thermal conditions, threat risk, threat magnitude, and intrinsic habitat characteristics.

- 2 (Chapter 4). *How do lizards respond to trade-offs among potential natural stressors?* For a better understanding of behavioural stress response, how lizards resolve potential trade-offs among the above factors (thermal conditions, threat risk, threat magnitude, and intrinsic habitat characteristics) was also tested.

- 3 (Chapters 5 and 6). *Does corticosterone have dose-dependent effects at a behavioural level?* In order to answer this question, the mating behaviours of lizards with high and moderate corticosterone increased levels were investigated.

- 4. (Chapters 5 and 6). *Do corticosterone behavioural effects change with the social context?* To answer this question two different approximations were used:

-4. a. I investigated dose-dependent corticosterone effects on intra- and intersexual behaviour and mating success of male common lizards in an intrasexual competition context (*Chapter 5*). Furthermore, the potential existence of adaptive behavioural strategies in response to different corticosterone levels (*i.e.* doses) was investigated.

-4. b. Dose-dependent corticosterone effects on female-male behavioural interactions and female mate choice in an intrasexual competition free context were investigated (*Chapter 6*).

- 5. (Chapter 6). *Does female mate choice depend on the context? Do male corticosterone levels play a role in that context?* Finally, I investigated if female mate choice is context dependent by testing females with different previous mating history mating decisions by sequentially presenting males with different corticosterone levels to the females.

Chapter 3

3 General methods

Model Species

General description, activity and reproduction

The common lizard (*Lacerta vivipara* Jacquin 1787) is a small ground-dwelling lizard of the family Lacertidae (Sauropsida: Squamata). Males' snout-vent-length (SVL) may measure up to 65 mm and females' SVL up to 75 mm. The tail's length is from 1.1 to 2.3 times the head length in males, and from 1.1 to 2.5 times the head length in females (Salvador and Pleguezuelos 2002). Juveniles of the common lizard are melanic at hatching (Figure 3. 1. a.) and start developing adult coloration during their first year of life (Vercken and Clobert 2008). Adult s' dorsum coloration is basically brown with a variable banded pattern (Figure 3. 1. b.). Adult's males exhibit ventral coloration that ranges from white to yellow and orange (Fitze *et al.* 2009), and in the Pyrenees, females exhibit only white ventral colours (Vercken *et al.* 2007b; San-Jose 2012). Melanic individuals have been described from one Pyrenean population but their frequencies are low (San-Jose *et al.* 2008).

Males emerge from hibernation between February and April approximately one month earlier than females, when the soil temperature reaches between 4ºC and 9º C and the maximum air temperature reaches between 12ºC and 20ºC (Salvador and Pleguezuelos 2002). Lizards go back to hibernation between September and November (Roig *et al.* 2000; Salvador and Pleguezuelos 2002). They spend hibernation in winter burrows usually situated below low bushes at a depth between 2 and 10 cm. During hibernation this species shows a very high freeze tolerance and it is capable of surviving even in a super-cooled state at -3ºC for at least three weeks (Voituron *et al.* 2004). Their daily activity starts when the body temperature (T_b) reaches 15-20º C. The common lizard is an accurate and effective thermoregulator (Van Damme *et al.* 1987; Gvozdik 2002). At low T_b it dedicates more time to basking in

order to increase their T_b by reducing the time spent looking for preys (Salvador and Pleguezuelos 2002). At low T_b they also reduced their movement capacity and their prey localization efficiency (Salvador and Pleguezuelos 2002). Their T_b goes from 16.2 to 37.8ºC (Salvador and Pleguezuelos 2002) but its preferred T_b goes from 30.7 to 34.7º C for males and form 31.9 to 37.4º C for females (Herczeg *et al.* 2006). It has been observed that male common lizards life expectancy is around 4 years. However, females may live for 6-7 years or even more (Massot *et al.* 2011).

The common lizard diet is based on small invertebrates depending on the environmental availability (Heulin 1986). It mainly preys on heteroptera and araneae (Avery 1962), but also on different animal groups, including other insects and non-insect taxa (oligochaeta, isopoda, gastropoda and myriapoda; Avery 1962; Koponen and Hietakangas 1972; Salvador and Pleguezuelos 2002).

The common lizard has fine chemoreceptive capabilities allowing it to distinguish between predatory and non-predatory chemical cues (Thoen *et al.* 1986; Van Damme *et al.* 1990). Among the common lizard depredators we find reptiles (*Coronella austriaca* or *Vipera* sp.), birds (*Asio flammeus*, *Athene noctua*, *Circus cyaneus*, *Coracias garrulous*, *Erithacus rubecula*, *Falco tinnunculus* and *Turdus* sp.) ands mammals (*Mustela erminea* or *Sus scrofa*) depending on each population (Salvador and Pleguezuelos 2002).

Males emerge from hibernation approximately 1 month earlier than females, to allow sperm maturation (Courty and Dufaure 1980; Roig *et al.* 2000). The common lizard is one of the few reptiles that exhibit reproductive bimodality (Roig *et al.* 2000). There are oviparous populations in the Cantabric Mountains, Pyrenees and Aquitaine (Lantz 1927; Surget-Groba *et al.* 2001). More recently, oviparous populations have also been found in Slovenia, in Northeastern and Northwestern Italy and in the Carnic Alps in Southern Austria (Ghielmi *et al.* 2001). Viviparous populations occur from central France and the British Isles up to Scandinavia and Russia (Surget-Groba *et al.* 2001).

The common lizard is a polygynandrous species (Fitze *et al.* 2005) being the number of female partners higher than the number of male partners. Female clutches are sired by an average of two different males but males may sire clutches of an average of four different females (Fitze *et al.* 2005). The clutches consist of 1 to 13 eggs that are laid after 3 to 6 weeks of gestation depending on the latitude and altitude (Pérez-

Mellado 1998; Laloi *et al.* 2004). In viviparous populations, the laid eggs have a soft shell and juveniles hatch few hours after egg laying (Fitze *et al.* 2005). However, in oviparous populations, the eggs are calcified and juveniles hatch after an incubation period of 30 to 50 days (Heulin *et al.* 1994). Usually, females may produce two clutches per year but in low altitude populations they may lay even three (just one in high altitude populations; Heulin *et al.* 1994). Juveniles are able to reproduce after the first hibernation in low altitudes but after the second or third hibernation in high altitudes (Heulin *et al.* 1994). However, sexual maturity is quite dependent on body size (see Massot *et al.* 2011).

Mating happens immediately after female emergence. During mating season, males aggressively chase away other males to ensure access to females (Heulin 1988) and sometimes they even fight for them. This suggests that male-male competition (intrasexual competition) for access to females exists, which is common in reptiles (Tokarz 1995) and may determine male fitness (Fitze *et al.* 2005; Fitze *et al.* 2007). In addition, qualitatively better males (bigger males, with better body condition) are more likely to reproduce (Fitze *et al.* 2007; Fitze *et al.* 2010) and thus female mate choice also plays an important role in common lizard mating. Females are able to resist male copulation attempts (Fitze *et al.* 2005; Fitze *et al.* 2010), and there is direct evidence that female mate choice depends on the mating order (Fitze *et al.* 2010) and on the costs of reproduction (Fitze and Le Galliard 2008) and thus, is context dependent. It has also been shown that male sexual harassment may influence females mate choice and therefore, male reproductive success (Fitze *et al.* 2005). Therefore, both male and female mate choice of common lizards are affected by a complex multifactorial interaction.

The fine chemoreceptive common lizard capabilities also allow them to distinguish among conspecifics of different levels of relatedness or familiarity (Léna *et al.* 2000). Therefore, chemoreception is an important social tool for common lizards. It is still generally accepted that the common lizard is a 'social' species (see for example Cote *et al.* 2008). For example, it is known that juveniles with poor body condition prefer the shelter with chemical cues from conspecific adult males because even when that mean potential intraspecific competition, an occupied patch also mean good quality territory and they might not be able to afford poor territory conditions (Aragón *et al.* 2006). However, several studies indicate that social

tolerance is in fact considerably lower than previously believed (Cote *et al.* 2007). First, the common lizard is a highly density-dependent species (Le Galliard *et al.* 2005), intra- and intersexual selection are important fitness determinants (Fitze *et al.* 2005; Le Galliard *et al.* 2005; Fitze and Le Galliard 2008; Le Galliard *et al.* 2008; Fitze *et al.* 2010), and aggression among adult males is higher in high-density populations, independently of the breeding season (Vercken and Clobert 2008). Second, agonistic behaviour (aggression and avoidance) and social stress among common lizard females has been documented during the early gestation and post-gestation period (Vercken and Clobert 2008), potentially affecting survival (Cote *et al.* 2006) and behaviour (Fitze *et al.* 2009), and third, ferocious fights occur between males over access to females (Fitze unpublished data).

Figure 3. 1. The common lizard. a. Juvenile common lizard. **b.** Adult common lizard (pictures by L. M. San-Jose).

Habitat, distribution and populations of study

The common lizard inhabits peat bogs and moist heath land (Massot *et al.* 1992). The principal environmental factor limiting the common lizard distribution is humidity (Ceirans 2007) because of their highly permeable, and thus very susceptible to humidity changes, skin (Grenot *et al.* 1987; Grenot and Heulin 1990). Humidity and water needs may even constrain growth and survival of the common lizards (Grenot *et al.* 1987; Lorenzon *et al.* 1999; Lorenzon *et al.* 2001).

The common lizard has a very wide distribution range all across Eurasia, from Ireland and the Northwestern Iberian Peninsula in the west, to Japan in the east, and from Scandinavia in the north, to Northern Iberian Peninsula and Macedonia in the south (Figure 3. 2. a.). In the Iberian Peninsula from where the populations used in this

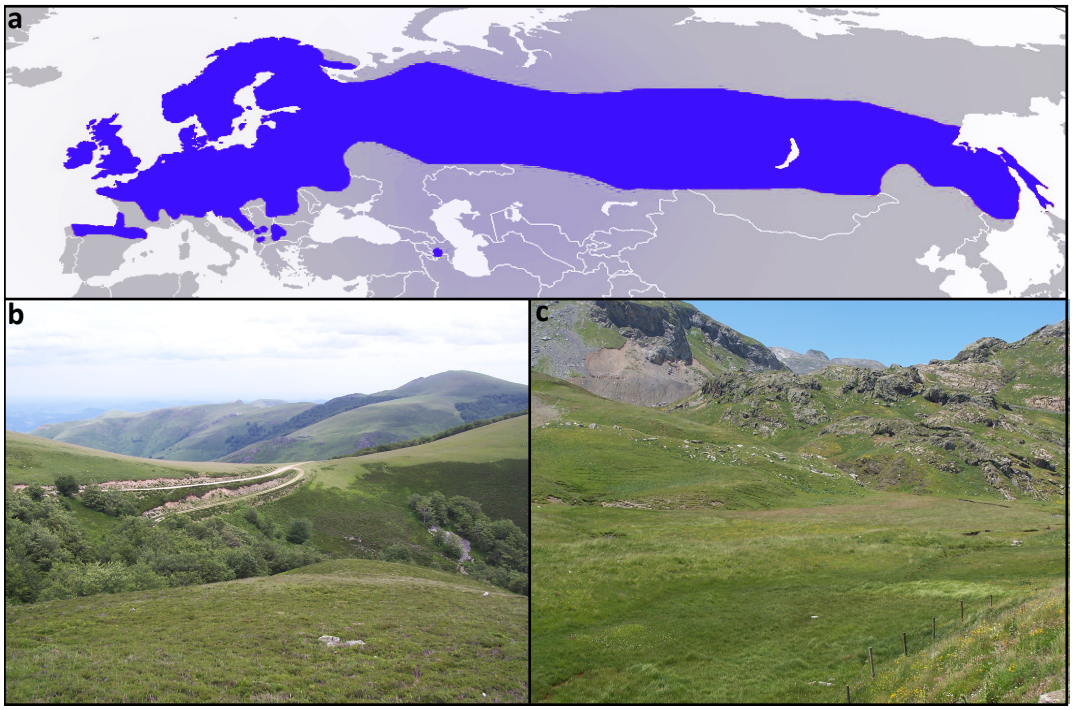

Figure 3. 2. Distribution and habitat of the common lizard. a. World distribution of the common lizard. **b**. Typical habitat of the common lizard in the Pyrenees (Roncesvalles, Navarra, Spain). **c**. Typical habitat of the common lizard in the Pyrenees (Somport, Huesca, Spain).

thesis came, its geographic distribution is restricted to mountainous habitats and lowlands of Atlantic influence being absent where more xeric conditions prevail (Pérez-Mellado 1998). More precisely, we used lizards from Roncesvalles, Navarra (Western Pyrenees, Spain, 1062 m a. s. l., 43º1'17.72"N, 1º19'13.5"W; Figure 3. 2. b.) and from Somport, Huesca (Western Pyrenees, Spain, 1640 m a. s. l., 42° 47' 45.88'' N, 0° 31' 32.77'' W; Figure 3. 2. c.). Both populations are located in mountain passes with quite similar vegetation consisting mainly of gorse (*Ulex* sp.) and heather (*Erica* sp.)

Stress and corticosterone effects on the common lizard

The common lizard as a reptile produces the glucocorticoid corticosterone during its stress response. Corticosterone levels have been measured in captive female common lizards (Dauphin-Villemant and Xavier 1987; Meylan *et al.* 2003). Basal corticosterone levels in captivity were between 14.4 ± 2.3 ng/ml (Dauphin-Villemant and Xavier 1987) and 21.64 ± 4.5 ng/ml (Meylan *et al.* 2003) but increase until 50.8 ± 9.2 ng/ml after 1 hour of confinement (Dauphin-Villemant and Xavier

1987). Corticosterone levels show a unimodal daily rhythm that is correlated with the rhythm of activity and it is constant over seasons except during vitellogenesis when the levels are slightly lower (Dauphin-Villemant and Xavier 1987). Corticosterone levels have also been studied during artificial hibernation (the levels increase when lizards are first transfer to hibernation conditions; Dauphin-Villemant *et al.* 1990). In addition, female corticosterone levels tend to be lower than male levels, and tend to be higher in gravid females than in non-gravid females (Meylan *et al.* 2003). Recently, wild female common lizards corticosterone levels have been studied in order to understand its relation with lizards' age. However, no age effect was found (Massot *et al.* 2011).

Many effects of stress and increased corticosterone levels have been reported in the common lizard. On one hand, Oppliger *et al.* (1998) studies the relationship between environmental stress and blood parasites. They found that stressed lizards had higher corticosterone levels and more blood parasites than non-stressed lizards. Meylan *et al.* (2010) studied the effect of increased corticosterone levels on physiology of gravid common lizards among different immune contexts. They found that increased corticosterone levels impaired clutch success and have negative effects on juveniles' body condition and size. However, Meylan *et al.* (2010) found that increased corticosterone levels had positive effects on physiological performance. The behavioural effects of increased corticosterone levels have also been studied. As expected from results in other species, corticosterone produced an increase in energy expenditure and food ingestion, and even modified the partition of the time dedicated to different behaviours: common lizards with increased corticosterone levels emerged earlier in the morning, were less immobile and dedicated more time to basking than lizards with lower corticosterone levels (Cote *et al.* 2006). However, lately it was shown how the behavioural modifications induced by high corticosterone levels depended on food availability. Under low food availability some of the behavioural and physiological modifications induced by high corticosterone levels might incur in high energetic costs and thus, some stress-related behaviours are inactivated in order to save energy (Cote *et al.* 2010a). Well-fed common lizards with increased corticosterone levels were active earlier in the morning, and increased the time spent basking but food-deprived lizards did not show those behavioural modifications (Cote *et al.* 2010a). In addition, one of the most surprising effects of high corticosterone levels

found on common lizards is that they positively affects male survival through its action on physiology and behaviour (Cote *et al.* 2006).

There is quite a great amount of studies that explore the mechanism underlying corticosterone prenatal exposure influence on juvenile common lizards phenotype. First, prenatal exposure to high corticosterone levels seems to be a mechanism affecting dispersal in common lizards. It was found that a prenatal exposure to corticosterone reduced dispersion and increased attraction for mothers' odour in juveniles common lizard (de Fraipont *et al.* 2000). Meylan *et al.* (2002) show that prenatal exposure to corticosterone decreased juveniles' dispersal behaviour if they came from from mothers in better body condition, while dispersion increased with juveniles' body condition. In addition, Meylan *et al.* (2004) also found that prenatal corticosterone exposure reduced older mothers' offspring dispersal and increased younger mothers' offspring dispersal. With respect to prenatal corticosterone exposure and juveniles' dispersal, Vercken *et al.* (2007a) conducted an experiment in which they modified timing and duration of the prenatal exposure in gravid female common lizards. They found that the duration of prenatal exposure to corticosterone affected juveniles' dispersal strategy depending on the clutch sex ratio: prolonged exposure during pregnancy (during the whole gestation) enhanced philopatry behaviour in female-biased clutches while a short prenatal corticosterone exposure (during just two weeks of gestation) promoted juveniles dispersal. When investigating the effect of prenatal exposure to corticosterone on dispersal-dependent behaviours of juvenile common lizards it was found that prenatal exposure to corticosterone decreased juvenile sprint speed and motivation to run (Meylan and Clobert 2004). In addition, Meylan *et al.* (2009) treated the mothers with corticosterone during the late gestation and tested juveniles' behaviour at birth and 10 months later. They found that a prenatal exposure to corticosterone enhances juveniles' activity but they did not find any other effects on other dispersal-dependent behavioural traits measured such as social or foraging behaviours. Therefore, the previously mentioned investigations make clear that prenatal corticosterone exposure influences dispersal behaviour of juvenile common lizards and that the effect depends on the interaction of many factors such as mother or clutch traits. However, what it is still not clear is what dispersal-dependent behavioural traits are exactly modified in order

to affect juveniles' dispersion decisions.

Prenatal exposure to corticosterone not only may affect juveniles behaviour, it may also modify some juvenile morphological characteristics (decreased juveniles' size, body condition and growth, Meylan and Clobert 2005). Vercken *et al.* (2007a) found that an early exposure (during the first two weeks of gestation) to corticosterone during pregnancy affected negatively juveniles' size, weight and body condition as well. However, Uller *et al.* (2005) did not found any effect of maternal stress (*i.e.* prenatal offspring exposure to corticosterone) on offspring sexually dimorphic morphology. Uller and Olsson (2006) also investigated the direct effect of corticosterone on offspring development by a surgical procedure that ended up with the injection of corticosterone directly in the eggs of common lizard females before clutch laying. They found that corticosterone-treated offspring did not show impaired development or either lower body size or condition than control offspring. However, prenatal corticosterone treatment produced abnormal antipredator behaviours in the offspring. Furthermore, Belliure *et al.* (2004) studied prenatal effects of corticosterone on activity and thermoregulation of new hatched juvenile common lizards as well. Prenatal exposure to corticosterone decreased the percentage of time spent moving of juveniles. However, they also found a modulating effect of sex and maternal condition on juveniles' behaviour: prenatal exposure to corticosterone increased scratching activity in female juveniles and in juveniles from larger mothers (Belliure *et al.* 2004). Consequently, other behaviours apart from dispersion are also influenced by a prenatal exposure to corticosterone on the common lizard. In addition, Meylan and Clobert (2005) found that a prenatal exposure to corticosterone increased juveniles' survival, which suggest a long-term adaptive effect of the prenatal corticosterone exposure. Finally, clutch quality is affected by prenatal exposure to corticosterone as well. Meylan *et al.* (2002) found that it produced a higher number of dead juveniles and delayed clutch laying.

Given that that a prenatal exposure to corticosterone has a great influence on juveniles' dispersal behaviour, there are also some studies that investigated if a postnatal exposure to corticosterone in recently born juveniles may also affect dispersion in the common lizard. Belliure *et al.* (2004) found that, as when the exposure to corticosterone was at a prenatal stage, only juvenile females increased their scratching activity when corticosterone was

delivered at a postnatal stage. Therefore, there is a modulating effect of sex on the effects of a postnatal exposure to corticosterone as well. Meylan *et al* (2002) also investigated the role of body condition and stress on dispersal at a postnatal stage. However, they did not found any effect of postnatal corticosterone exposure on juvenile dispersal propensity.

Regarding to the effect of stress and corticosterone on other phenotypic traits, it is believed that they affect carotenoid-based coloration and thus stressed animals usually exhibit reduced carotenoid based colorations (Milinski and Bakker 1990; Brawner *et al.* 2000; Meylan *et al.* 2007). However, in the common lizard it has been shown that high corticosterone levels increased the carotenoid based colour (redness) of the belly (Fitze *et al.* 2009; Cote *et al.* 2010b). Nevertheless, this effect was again dependent on food availability and thus food-deprived lizards did not show that corticosterone colour enhancement (Cote *et al.* 2010b).

Other different approaches on the study of corticosterone effects have also been investigate. Some other authors had studied corticosterone effects in the common lizard at a biochemical level. Courty (1991) studied corticosterone effect on the expression of genes coding for proteins related to the sperm maturation process, and Martin and Xavier (1981) investigated the binding activity of specific steroid-binding plasma proteins (regulate the concentration of free steroids) for corticosterone. Finally, at a more strict physiological level, adrenal activity had also been studied in detail in the common lizard (Dauphin-Villemant and Xavier 1985; Dauphin-Villemant *et al.* 1988).

Laboratory Conditions

All lizards were captured by hand and brought to Instituto Pirenaico de Ecología (Jaca, Huesca) where they were measured and thereafter maintained in individual terraria (25 x 15 x 15 cm) under standardized conditions. Each individual terrarium had a shelter (10 x 10 x 5 cm), *ad libitum* water and was closed with a mesh. Experimental terraria were enlightened and heated by a bulb (40 W bulb, 8 am until 6 pm) that was suspended on one terrarium side 15 cm above the terrarium. A UV-light source (5% UVB, 30% UVA, Sylvania Reptistar; 12 pm until 2 pm) provided the UV component of natural sunlight. Each terrarium was sprayed with water twice per day in order to maintain humidity conditions.

Lizards were fed with moth larvae *Galleria mellonella* (Lepidoptera: Pyralidae) with a frequency that depends on each the experiments (for more details see *Specific material and methods* of *Chapters 4*, *5* and *6*). At the end of the experiment lizards were released in the place they were captured.

Corticosterone Treatment

To investigate the effects of moderate and high corticosterone elevation on intra- and intersexual behaviour (*Chapters 5* and *6*), we artificially increased lizards' corticosterone levels. For that propose we used three different treatments. Common lizards in which we wanted to produce a high corticosterone elevation (hereafter referred to as "Cort1.5") were treated with 4.5 µl of sesame oil mixed with corticosterone (1.5 µg of corticosterone Sigma C2505 per µl of oil). This dose corresponds to a 1:2 dilution of the concentration used by Meylan *et al.* (2003). To investigate dose dependent effects, we as well used a dilution of the former solution, which leads to moderate elevations (hereafter referred to as "Cort0.15"). Lizards belonging to that group were thus treated with 4.5 µl of a 1:10 dilution (0.15 µg of corticosterone per µl of oil). Finally, common lizards used as a control group (hereafter referred to as "Control") were treated with 4.5 µl of sesame oil only.

The consequences of the here-applied treatments on plasma corticosterone levels were measured in another experiment (article in preparation). In brief, under field conditions male basal corticosterone levels were on average 12.1 ± 3.1 ng/ml similar to the values previously reported (Dauphin-Villemant and Xavier 1987; Meylan *et al.* 2003). Due to confinement, basal levels in the laboratory were 99.9 ± 15.6 ng/ml (Control group), which is within the variation observed in other reptile species (Tyrrell and Cree 1998). After treatment application, corticosterone levels reached on average 119.1 ± 27.0 ng/ml in the Cort0.15 group and 194.5 ± 7.9 ng/ml in the Cort1.5 group. Both levels were above the corticosterone levels observed in the control group (+19.2%, +94.7%), they were below the highest corticosterone levels (-57.7%, -31.0%) observed for a mix of 3 µg corticosterone per µl (Meylan *et al.* 2003) and within the natural variation observed in other reptiles in response to a stressful stimulus (Tyrrell and Cree 1998).

Ethical Standards

The experiments performed comply with the current law of Spain and with the ABS/ASAB guidelines for ethical treatment of animals.

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Chapter 4

4

Behavioural response to natural stressors: Multifactorial trade-off in microhabitat selection between thermal conditions, threat risk, and intrinsic habitat characteristics

Gonzalez-Jimena V, Fitze P , *in preparation*

Abstract

Optimal strategies in microhabitat selection may depend on intrinsic habitat characteristics, threat risk, threat magnitude, and complex trade-offs among them. We manipulated these factors and investigated the occurrence of trade-offs in microhabitat selection and the relative importance of different behavioural responses. We exposed common lizards to predator, conspecific, or control odour (*i.e.* different levels of threat magnitude) on one terraria side and to own odour on the other terraria side, reflecting different levels of threat risk. Two habitats differing in intrinsic characteristics were simulated: *trade-off* habitat (warmer microhabitat could only be selected when selecting habitat containing experimental odour), and *no trade-off* habitat (experimental odour could be avoided and warmer microhabitat simultaneously selected). We show that microhabitat selection depended on intrinsic habitat characteristics, threat risk, threat magnitude, and complex trade-offs among them. Lizards exposed to predator or conspecific odour did not differ in microhabitat selection, suggesting that conspecific competition may be more important for non-territorial species than previously believed. There existed fine-tuned responses, which followed a behavioural hierarchy ranging from low-cost behaviours conducted in low-cost situations, *i.e.* habitat avoidance in *no trade-off* habitats, to high cost behaviours conducted in high-cost situations, *i.e.* reduced thermal preferences, increased time spent in high threat risk habitat and increased movement when exposed to predator odour in *trade-off* habitat. Our results show that microhabitat selection, which may directly affect Darwinian fitness, is a complex task that depends on multifactorial integration of biotic and abiotic factors and complex trade-offs among them.

Keywords:

Chemical communication ◆ Conspecific avoidance ◆ Ectotherms ◆ *Lacerta vivipara* ◆Predator avoidance

Comportamiento en respuesta a factores estresantes de origen natural:
Selección de microhabitat en función del conflicto entre características termales, riesgo de posibles amenazas y magnitud de las mismas

Resumen

Las estrategias óptimas en cuanto a selección de microhábitats dependen por un lado de las propias características intrínsecas del hábitat y por otro del riesgo de posibles amenazas, la magnitud de la posible amenaza y también de complejos conflictos que surgen entre los anteriores factores. Se manipularon estos factores y se investigó la aparición de conflictos derivados de la selección de microhábitat y también la importancia relativa que tienen los comportamientos surgidos en respuesta. Se expuso a lagartijas de turbera a olores procedentes de un depredador, de individuos de la misma especie y a un olor control (es decir, a posibles amenazas de distinta magnitud). Estos olores experimentales estaban situados en un lado del terrario mientras que en el otro lado se mantenía el olor del propio individuo. Esta distinción entre el olor de los dos lados del terrario suponía dos niveles de riesgo distintos ante la amenaza representada por el olor experimental. A la vez, se simulaban dos hábitats que diferían en sus características intrínsecas: "hábitat con conflicto presente", en el que el microhábitat más cálido sólo podía ser seleccionado a la vez que se seleccionaba el olor experimental, y "hábitat sin conflicto presente" en el que el olor experimental podía ser evitado a la vez que se podía seleccionar el microhábitat más cálido. Se mostró que la selección de microhábitat depende de las propias características intrínsecas del mismo, del riesgo de posibles amenazas, de la magnitud de las mismas y de complejos conflictos entre estos factores. Las lagartijas expuestas al olor de depredadores o de individuos de su misma especie no mostraron diferencias en cuanto a la selección de microhábitat lo que sugiere que la competencia intraespecífica podría ser más importante de lo que se creía incluso en especies no territoriales. Por otro lado, se observó la aparición de una serie de comportamientos en respuesta a los conflictos surgidos muy precisos y que seguían una jerarquía que iba desde comportamientos de bajo coste en situaciones que no entrañaban alto riesgo para el individuo (como evitar un hábitat concreto en el caso de los "hábitats sin conflicto presente"), hasta comportamientos de alto coste en situaciones que si entrañaban un alto coste para el individuo (como reducir las preferencias por los hábitats más cálidos, aumentar el tiempo en zona de riesgo y aumentar la actividad en respuesta al olor de un depredador en los "hábitats con conflicto presente"). Los resultados muestran que la selección de microhábitats, que puede afectar directamente a la

eficacia biológica de los individuos, es un tema muy complejo que depende a su vez de la integración de multitud de factores tanto bióticos como abióticos y de los complejos conflictos que pueden surgir entre ellos.

Palabras clave:

Comunicación química ◆ Ectotermos ◆Evitación de depredadores ◆ Evitación de individuos de la misma especie ◆ *Lacerta vivipara*

Introduction

Microhabitat selection is a crucial decision for animals that depends on food availability (Anderson 2007; Naxara, *et al.* 2009), refuge availability (Williams and McBrayer 2007; Fox and Monamy 2007), predation risk (MacKenzie and Greenberg 1998; Sapir *et al.* 2004; Webb *et al.* 2009), intra-specific competition (Stamps 1991; Fox and Monamy 2007), and thermal conditions (Oneill and Oneill 1988; Goodfriend *et al.* 1991; Hertz *et al.* 1994). Inadequate microhabitat selection may have negative consequences due to reduced food intake, increased intra- and inter-specific competition, increased physiological stress, and increased mortality, all of them potentially leading to reduced Darwinian fitness (Oneill and Oneill 1988; Huey 1991). Microhabitat choice is thus a complex task since several factors need to be integrated at the same time, potentially leading to trade-off situations where a given microhabitat is optimal for one characteristic, but suboptimal with respect to other characteristics. How animals resolve such trade-offs is a fascinating area of research, but despite the many studies investigating habitat selection (*e.g.* Downes 2001; Whitehead *et al.* 2002; Webb *et al.* 2009; Wang *et al.* 2010), knowledge about how potential trade-offs are resolved is still scarce.

Microhabitat selection with respect to thermal conditions has attracted much research, since thermal conditions are important for most animals ranging from unicellular organisms to insects and mammals. They are of special importance for ectotherms, which exhibit low basal metabolic rates that are insufficient for physiological thermoregulation (Bennett 1983). Therefore, ectotherms essentially adjust optimal body temperature (T_b) through microhabitat selection (Stevenson 1985; Huey *et al.* 1989). In general, low T_b reduces metabolic rate and results in low physiological performance in terms of speed and stamina, while high T_b increases metabolic rate and enhances physiological performance (Moberly 1968; John-Alder and Bennett 1981; Bennett 1983; Chen *et al.* 2003; Pinch and Claussen 2003). This shows that optimal T_b depends on the conducted behavioural task (Martín and López 1999; 2001; 2010) and it suggests that ectotherms should adjust T_b accordingly (Regal 1970; Van Damme *et al.* 1990a). Since in many ectotherms a positive correlation between time exposed to a heat source and T_b has been demonstrated (*e.g.* Díaz *et al.* 1996, Van Damme *et al.* 1987), optimal T_b is mainly achieved by adjusting microhabitat selection and the time spent in a given habitat. For

example, in the presence of predation risk, ectotherms should prepare for a potential interaction with a predator and increase their T_b in order to improve escape ability. As a consequence, they should select warmer microhabitats more frequently and spend more time in these habitats. Habitat choice may as well depend on the magnitude of threat, as predicted by the threat-sensitivity hypothesis (Helfman 1989). While achieving optimal T_b may be crucial to survival when facing a predator, it may be of reduced importance when facing a competitor, since encountering a competitor may lead to lower fitness, but rarely to death (see Krebs and Davies 1993). Microhabitat selection may as well depend on intrinsic habitat characteristics, *i.e.* characteristics that define the habitat and that may impose additional costs (*e.g.* food and shelter availability or predators lethality). In this case, ectotherms may have to make a compromise between avoiding predation risk, thermal preferences and intrinsic habitat characteristics. As a consequence they may select warmer habitats less frequently than predicted by predation risk (Huey and Slatkin 1976). This suggests that microhabitat selection is the result of multifactorial integration based on threat risk, threat magnitude, and intrinsic habitat characteristics. Although many studies investigated microhabitat selection and thermal preferences in relation to threat risk (Martín and López 1999; 2001; 2010; Van Damme *et al.* 1990a; Herczeg *et al.* 2008; Webb *et al.* 2009), experimental evidence for the existence of multifactorial integration and more specifically for the existence of a trade-off between thermal preferences and threat avoidance is very limited.

Here we experimentally investigated whether microhabitat selection of the common lizard (*Lacerta vivipara* Jacquin, 1787) is the result of multifactorial integration based on thermal preferences, threat risk, threat magnitude, and intrinsic habitat characteristics. We further tested how lizards resolve trade-offs among these factors. We manipulated threat risk and threat magnitude, and submitted lizards to two habitat types differing in intrinsic habitat characteristics. We took advantage of the high olfactory capabilities of lizards (Léna and Fraipont 1998; Aragón *et al.* 2001; Downes 2002), exposing them on one side of their terrarium to threats of varying magnitude, namely odour of predators (high threat risk), conspecifics (female and male odour, lower threat risk), or no additional odour (control, no threat risk), while on the other side their own odour was present (*i.e.* no threat presence). Two habitats with different intrinsic characteristics were simulated. Half of the lizards

of each threat magnitude group were exposed to a *trade-off* habitat, *i.e.* to an habitat in which lizards could not avoid direct contact with the experimental odour when staying on the warmer side (experimental odour and heat were provided on the same terraria side), or to a *no trade-off* habitat, *i.e.* to an habitat in which lizards could simultaneously avoid direct contact with the experimental odour and select a warmer microhabitat (experimental odour and heat were provided on two different terraria sides). According to the threat-sensitivity hypothesis (Helfman 1989), and together with knowledge on strategies adopted by lizards in the presence of threat risk (*e.g.* Rand 1964; Amo *et al.* 2004a; Amo *et al.* 2008), we predicted that *(a)* lizards should generally avoid threat risk to increase Darwinian fitness. Thus, lizards exposed to predator or conspecific odour should spend less time on the side with experimental odour compared to control lizards. Given that higher T_b has been shown to increase the chance of a successful escape (Bennet 1983; Chen *et al.* 2003; Pinch and Claussen 2003), we as well predicted that *(b)* lizards exposed to predator or conspecific odours should spend more time on the warmer terraria side to prepare for potentially stressful situations (Van Damme *et al.* 1990a). We further predicted that *(c)* the behavioural reactions (locomotor activity, appeasement and exploratory behaviours) depend on the threat magnitude and therefore that more acute behavioural reactions should be observed in lizards exposed to predator odour than in lizards exposed to conspecific odour, and we predicted differing responses with respect to intra- and inter-sexual competition. In addition, we predicted that intrinsic habitat characteristics affect multifactorial integration. For *no trade-off* habitat we predicted that lizards behave as predicted above (prediction *(a)*, *(b)*, *(c)*). However, in t*rade-off* habitat, we predicted the existence of a trade-off between allocating time to warmer microhabitat and threat avoidance and thus, that *(d) trade-off* lizards (compared to *no trade-off* lizards) reduce the time spent in warmer microhabitat to avoid threat risk and that *(e) trade-off* lizards will increase the time spent in the presence of threat risk in order to be on the warmer side.

Specific Material and Methods

Experimental design

Forty-eight common lizards were used for the experiment: 24 adult males, and 24 adult females. All lizards were captured by hand between 9 and 11 August 2007,

after the reproductive period, in Roncesvalles population (Navarra). Lizards were maintained under standardized conditions (see *Chapter 3*) and fed every two days with moth larvae (0.3-0.35 g *Galleria mellonella*, Pyralidae).

At the start of the experiment (day 1), lizards were transferred to new terraria, hereafter referred to as 'experimental terraria', containing a shelter and *ad libitum* water. On day 2, male and female lizards were exposed to one of four different experimental odours corresponding to different threat magnitudes (n = 6 males + 6 females / threat magnitude, Figure 4. 1): *(a)* adult female *L. vivipara* odour (F), *(b)* adult male *L. vivipara* odour (M), *(c)* predator odour (P), and *(d)* no additional odour (control, C). Threat risk was manipulated by lining each experimental terrarium on one side with absorbent paper containing own odour (Figure 4. 1) and on the other side with one of the four different experimental odours (Figure 4. 1). To create differing thermal conditions a heat and light source was situated on one side of the terrarium. There were significant differences in thermal conditions (mean ± SE: warmer side = 35.3 ± 0.4ºC, cooler side = 34.1 ± 0.5ºC; matched pair test: t_{11} = 6.384, P < 0.001), and temperatures of both microhabitats were within the range of preferred T_b reported for this species (Herczeg *et al.* 2006). To manipulate intrinsic habitat characteristics, we manipulated habitat type by submitting half of the lizards (n = 24) to a *trade-off* habitat where the heat source was provided on the side of the experimental odour. In this habitat type, lizards could either avoid threat risk, but not select the warmer microhabitat, or could select the warmer microhabitat, but not avoid threat risk (Figure 4. 1). Therefore they were exposed to a trade-off situation. The other half of the lizards were exposed to a *no trade-off* habitat where the heat source was provided on the side of the own odour, allowing them to simultaneously select the warmer microhabitat and avoid threat risk (Figure 4. 1).

Half of the lizards of each treatment combination (threat magnitude x habitat type), and sex were exposed to the heat source on the right side and the other half on the left side of the experimental terraria. Furthermore, half of the lizards of each treatment combination and sex were assigned to one of two blocks, to ensure that all individuals could be handled under the same standardized conditions. For the first block the experiments started on 02-Sept-2007 and for the second block the day after the first block of experiments ended. There were no significant differences

between treatment combinations, heat source position, and blocks in snout-vent length (SVL), body mass, body condition and date of capture (all $P > 0.5$).

Detailed experimental procedures

On day 1, animals used as odour donors were transferred to new individual terraria (45 x 25 x 25 cm) hereafter referred to as 'donor terraria'. Two individuals per threat magnitude were used as odour donors for the entire experiment. For the control group the same protocol was used but no animals were added. To simulate the presence of a predator we used *Vipera seoanei* Lataste, 1879, which is a common predator of *L. vivipara* in the Roncesvalles population. Donor terraria (n = 8) were layered with absorbent paper (42 x 23 cm, 60 g m^{-2}, ALBET 80250452) contained a heat and light source (40 W bulb), and *ad libitum* water. After 24 hours

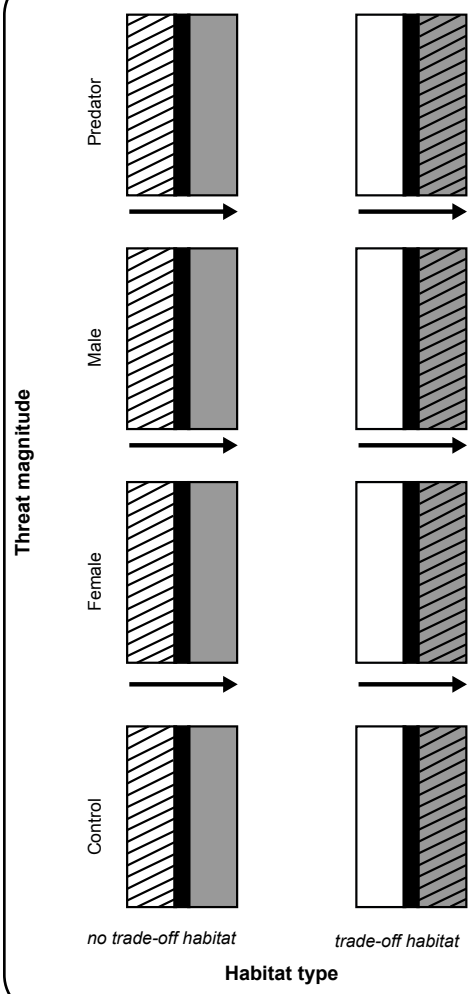

Figure 4. 1. Experimental design. We manipulated threat risk, threat magnitude, and intrinsic habitat characteristics using eight different experimental arrangements presented in this figure. All terraria contained two halves of absorbent paper, one containing own odour (white colour) and the other containing experimental odour (grey colour), representing differences in threat risk. To analyse microhabitat selection a middle area was defined, which is represented in black. One terraria side was illuminated and heated with a bulb corresponding to the warmer side (hatched half), while the other corresponded to the cooler side (unhatched half). Four levels of threat magnitude were simulated. Control odour (C) corresponds to no threat, conspecific odour (F = female, M = male) corresponds to increased threat, and predator odour (P) corresponds to the highest level of threat. Habitat type was manipulated by submitting lizards to a *trade-off* habitat (right column) or a *no trade-off* habitat (left column). In the *trade-off* habitat the experimental odour was on the same side as the heat and light source (grey and hatched half), while in the *no trade-off* habitat they were on different sides. For F, M, and P a horizontal arrow indicates increasing threat risk. The total sample size was N = 48, and N was the same for each of the eight arrangements (N = 6; 3 males + 3 females).

absorbent papers of the donor were removed (day 2). Each donor absorbent paper was cut with clean scissors into six pieces and kept in separate clean hermetic plastic bags during a standardized amount of time before they were used.

At the same time (day 1), experimental lizards were introduced into experimental terraria (25 x 15 x15 cm), which were layered with two absorbent paper halves (7 x 23 cm). Experimental terraria were enlightened and heated by a bulb (40 W bulb, 8 am until 6 pm) that was suspended on one terrarium side 15 cm above the terrarium. A UV-light source (5% UVB, 30% UVA, Sylvania Reptistar; 12 pm until 2 pm) provided the UV component of natural sunlight. After 24 hours of contact with the experimental lizards, half of the absorbent paper was removed and a randomly chosen absorbent paper of a donor terrarium was added instead. As a result, one half of each experimental terrarium was layered with absorbent paper containing own odour and the other half with absorbent paper containing donor odour. Once the experimental terraria were prepared, lizards were introduced to the midline and allowed to adapt to the new environment for 15 minutes. Lizard behaviour was then recorded during eight minutes.

All terraria (experimental and donor) were previously cleaned with alcohol and handled with clean plastic gloves only. All absorbent papers were clean and handled using alcohol-cleaned tweezers and gloves to prevent contamination. All terraria were enclosed with a mesh to avoid animals from escaping and potential contamination by insects. The positions of donor, control, and experimental terraria were randomly assigned and experimental terraria were spatially separated from the other terraria to prevent lizards from perceiving experimental odours before the start of the behavioural experiment.

Behavioural observations

Behaviour was quantified by the same naïve observer. In order to investigate multifactorial integration based on threat risk, threat magnitude, and intrinsic habitat characteristics we quantified the following behaviours in seconds:

- **1**. Amount of time moving. The variable quantifies locomotor activity and reflects the intensity of exploratory behaviour (Braña 2003).

- **2**. Amount of time trying to escape. Quantified behaviours including the time lizards stood in an upright position against the terrarium wall and simultaneously performed scratching movements with their forelegs (Thoen *et al.*

1986), and situations where lizards tried to burrow in the substratum using their forelegs (Van Damme *et al.* 1990a). Scratching behaviour is an indicator of individual stress (Vercken and Clobert 2008).

– **3**. Amount of time hiding. The time lizards spent inside the shelter or below the absorbent paper was quantified. The amount of time hiding is an indicator of threat perception, since prey often responds to predator presence by increasing refuge use (Sih *et al.* 1992).

We also registered the number of times a lizard showed one of the following behaviours:

– **1**. Number of tongue extrusions. The number of times a lizard rapidly extruded and retracted its tongue. It measures exploratory activity and reflects the strength of a lizard's response to a stimulus (Cooper and Burghardt 1990).

– **2**. Number of appeasement displays. We counted the number of times a lizard moved the entire tail or the tail tip rapidly from side to side and the number of times a lizard moved its forelimbs rapidly up and down (Thoen *et al.* 1986). Both behaviours are exhibited during social interactions and represent appeasement displays to avoid costly interactions (Punzo 2007).

Finally, we quantified microhabitat selection. We registered the amount of time (in seconds) spent on a particular terrarium side. For this purpose we defined three areas: experimental odour side (6.5 x 25 cm, 43.33% of the terrarium surface), middle area (2 x 25 cm, 13.33% of the terrarium surface), and own odour side (6.5 x 25 cm, 43.33% of the terrarium surface) and analysed the amount of time spent on the experimental or the own odour side (for details, see statistics).

Statistics

Analyses were run in R 2.9.0 (Free Software Foundation, GNU Project, Boston, MA, USA) and JMP IN 4. 0. 3 (SAS Institute). Model selection started with the full model, which included all parameters and their interactions, and the final model was determined using backward elimination. For all tests the significance level was set at $P \leq 0.05$ (two-tailed test). The assumptions of the applied models were tested (*e.g.* normality and homocedasticity of the residuals for ANOVAs). If these criteria were not met, transformations were applied.

We applied linear mixed effects models for time data and for count data we used generalized linear mixed models via PQL with Poisson

error distribution (Quinn and Keough 2002). Threat magnitude, habitat type, and sex were modelled as fixed factors and block and donor individual as random factors. Microhabitat selection was analysed using linear mixed models with the amount of time spent on a particular terraria side as dependent variable, threat magnitude, threat risk, habitat type, sex, and their interactions as fixed factors and lizard, block nested within lizard, and donor individual as random factors. In this model we considered only the amount of time spent on the experimental odour and the own odour side, while the amount of time spent in the middle of the terrarium (on average 27% of the time), was not included, given that it could be determined unequivocally (8 minutes – time spent on the experimental odour side – time spent on the own odour side), and thus its inclusion was redundant. In all above-mentioned analyses, block, donor individual, sex, and the interactions including these factors were not significant (all $P > 0.1$), therefore statistical results are not presented in the results section. To localize significant differences between factor levels we applied *post hoc* tests (Quinn and Keough 2002) and used sequential Bonferroni corrections to adjust α for the increased probability of obtaining statistical significance from multiple testing (Quinn and Keough 2002). The statistical power ($1-\beta$) for detecting significant treatment effects was for all models ≥ 0.62.

The here used behavioural variables were statistically independent, since no significant correlations existed among them (for all variables $r^2 < 0.061$, $P > 0.5$), with the exception of the amount of time moving and the amount of time trying to escape which were positively correlated ($r^2 = 0.45$, $P = 0.001$).

Results

Behavioural traits

The amount of time moving depended on a significant interaction between threat magnitude and habitat type (Table 4. 1, Figure 4. 2a). Individual contrasts showed that the amount of time moving was significantly higher in P lizards of *trade-off* habitat than in C, F, and M lizards of *trade-off* habitat (P *vs.* C: $F_{1,39} = 14.257$, $P_{adj} < 0.001$, P *vs.* F: $F_{1,39} = 19.946$, $P_{adj} < 0.001$, P *vs.* M: $F_{1,39} = 18.590$, $P_{adj} < 0.001$, Figure 4. 2a), and it was also higher than in C and P lizards of *no trade-off* habitat ($P_{trade\text{-}off}$ *vs.* $C_{no\ trade\text{-}off}$: $F_{1,39} = 16.740$, $P_{adj} < 0.001$, $P_{trade\text{-}off}$ *vs.* $P_{no\ trade\text{-}off}$: $F_{1,39} = 14.920$, $P_{adj} < 0.001$, Figure 4. 2a).

The amount of time hiding and the amount of time trying to escape

were not significantly affected by threat magnitude ($F_{3,42}$ = 0.945, P = 0.430; $F_{3,42}$ = 1.164, P = 0.335, respectively), habitat type ($F_{1,42}$ = 0.106, P = 0.747; $F_{1,42}$ = 0.132, P = 0.719, respectively), nor by their interaction (P > 0.05).

The number of tongue extrusions depended on threat magnitude (Table 4. 1) and no significant interaction between threat magnitude and habitat type existed (P > 0.05). Lizards exposed to predator odour showed a higher tongue extrusion rate than the other lizards (P *vs.* C: $F_{1,43}$ = 8.830, P_{adj} = 0.005, P *vs.* F: $F_{1,43}$ = 9.690, P_{adj} = 0.004, P *vs.* M: $F_{1,43}$ = 10.132, P_{adj} = 0.004, Figure 4. 2b) and no significant differences existed between C, F, and M lizards (P_{adj} > 0.05).

The number of appeasement displays significantly depended on threat magnitude and habitat type (Table 4. 1). P lizards showed significantly more appeasement displays than lizards of the other three groups (P *vs.* C: $F_{1,42}$ = 7.995, P_{adj} = 0.008, P *vs.* F: $F_{1,42}$ = 9.380, P_{adj} = 0.008, P *vs.* M: $F_{1,42}$ = 7.778, P_{adj} = 0.008; Figure 4. 2c), and lizards in the *trade-off* habitat (mean ± SE = 1.382 ± 0.563) showed more than lizards in the *no trade-off* habitat (mean ± SE = 0.515 ± 0.398; Table 4. 1). No significant interaction between threat magnitude and habitat type existed.

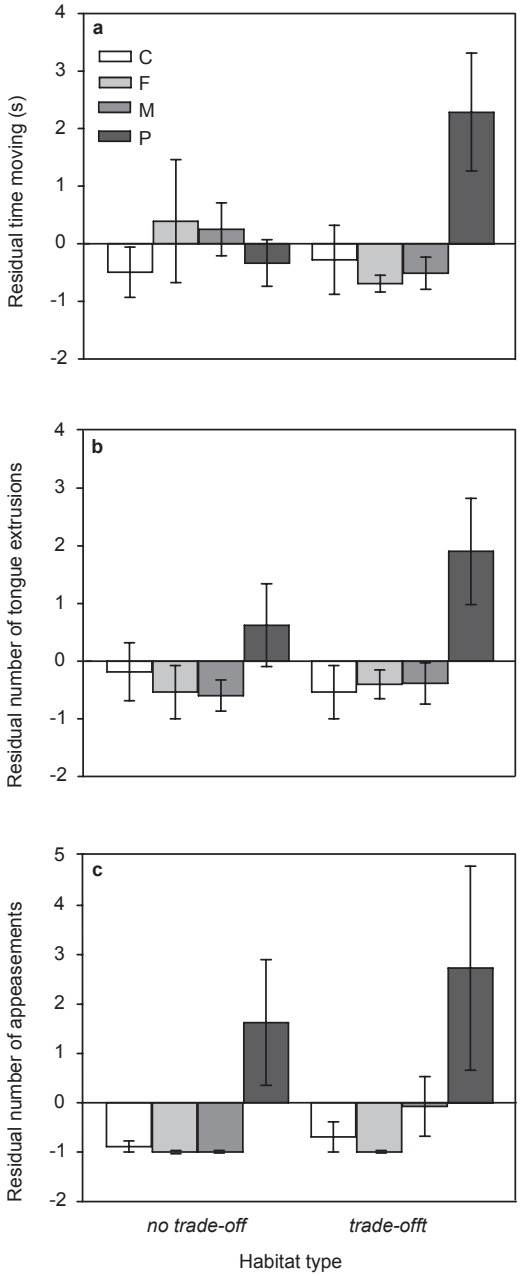

Figure 4. 2. Effects of threat magnitude (C = control, F = female, M = male, P = predator) and habitat type on (a) amount of time moving; (b) number of tongue extrusions; and (c) number of appeasement displays. Displayed residuals stem from a model including block as random factor. Bar shading corresponds to the four simulated threat magnitudes.

Table 4. 1. Treatment effects on: amount of time moving, number of tongue extrusions, number of appeasement displays, and time spent on a given terraria side.

Dependent variable	Parameter	*F* (*P*)	% v exp
Time moving	**Threat magnitude (TM)**	$F_{3, 39}$ = 3.655 (0.021)	**17.325**
	Habitat type (HT)	$F_{1, 39}$ = 3.590 (0.066)	5.673
	TM x HT	**$F_{3, 39}$ = 3.245 (0.032)**	**15.383**
Number tongue extrusions	**TM**	**$F_{3, 39}$ = 5.139 (0.004)**	**26.393**
Number appeasement displays	**TM**	**$F_{3, 42}$ = 5.436 (0.003)**	**26.090**
	HT	**$F_{1, 42}$ = 4.199 (0.047)**	**6.717**
Amount of time spent on a particular terraria side*	TM	$F_{3, 40}$ = 1.074 (0.371)	4.683
	HT	$F_{1, 40}$ = 0.005 (0.941)	0.008
	Threat risk (TR)	**$F_{1, 40}$ = 4.224 (0.046)**	**6.140**
	TM x HT	$F_{3, 40}$ = 1.503 (0.229)	6.554
	TM x HT	$F_{3, 40}$ = 0.563 (0.643)	2.454
	HT x TR	$F_{1, 40}$ = 0.227 (0.637)	0.330
	TM x HT x TR	**$F_{3, 40}$ = 4.973 (0.005)**	**21.687**

Results of general linear mixed models with Poisson error are shown, except for time spent on a particular terraria side, where results of a linear mixed effect model are shown. Model selection started with the full model. Non-significant terms were backward eliminated and results of the final model including test statistics, *P*-values, and percentage of variance explained are shown. Significant parameters are plotted in bold. *Applied transformation: $y^{½}$

Microhabitat selection

Microhabitat selection depended on a significant three-way interaction between threat risk, threat magnitude, and habitat type (Table 4. 1, Figure 4. 3). Control lizards spent more time on the cooler side, independent of habitat type (individual contrasts: *no trade-off* habitat: $F_{1,40}$ = 4.984, P_{adj} = 0.047; *trade-off* habitat: $F_{1,40}$ = 5.966, P_{adj} = 0.038; Figure 4. 3) and independent of the presence/ absence of own odour.

In the *no trade-off* habitat, lizards exposed to F, M, and P odours avoided threat risk (experimental odour side) and spent more time on the warmer own odour side (all individual contrasts, experimental odour side *vs.* own odour side: $F_{1,40}$ ≥ 13.585, P_{adj} ≤ 0.001, Figure 4. 3). There were no significant differences in the amount of time

spent on the warmer side between F, M, and P lizards (P_{adj} > 0.05) and lizards of all three groups spent more time on the warmer side than C lizards ($C_{warmer\ side}$ *vs.* $F_{warmer\ side}$: $F_{1,40}$ = 7.127, P_{adj} = 0.029, $C_{warmer\ side}$ *vs.* $M_{warmer\ side}$: $F_{1,40}$ = 12.503, P_{adj} = 0.006, $C_{warmer\ side}$ *vs.* $P_{warmer\ side}$: $F_{1,40}$ = 6.877, P_{adj} = 0.029, Figure 4. 3).

F, M, and P lizards of the *trade-off* habitat, did not spend significantly different amounts of time on the warmer side with threat risk (experimental odour) than on the cooler side without threat risk (all individual contrasts P_{adj} > 0.05). F, M, and P lizards of the *trade-off* habitat, spent significantly less time on the warmer side ($F_{1,40}$ = 5.584, P_{adj} = 0.039, Figure 4. 3), and tended to increase the time spent on the side with threat risk ($F_{1,40}$ = 2.481, P_{adj} = 0.119) compared to F, M, and P lizards of the *no trade-off* habitat.

Discussion

Microhabitat selection has important implications for animals since it may directly affect Darwinian fitness. While responses to a single factor, *e.g.* predator presence or thermal conditions, have been widely studied (Van Damme *et al.*, 1990a; Martín and López 1999; 2001; 2010; Herczeg *et al.* 2008; Webb, Pringle and Shine 2009), it is unclear how animals resolve multifactorial problems. Here, we tested whether behavioural decisions depend on threat risk, threat magnitude, and intrinsic habitat characteristics, and whether they followed additive or interactive patterns. Our results showed that *L. vivipara* adjusted locomotor behaviour, exploratory behaviour, and appeasement displays according to threat magnitude, and selected microhabitat according to a complex three-way interaction between threat risk, threat magnitude, and intrinsic habitat characteristics.

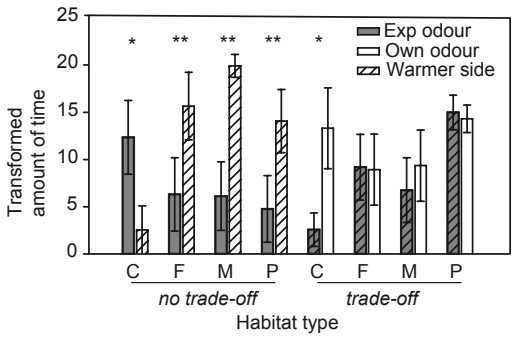

Figure 4. 3. Effects of threat risk (Exp odour = experimental odour side, *i.e.* with threat risk; Own odour = own odour side, *i.e.* without threat risk), threat magnitude (C = control, F = female, M = male, P = predator) and habitat type (*no trade-off* habitat, *trade-off* habitat) on microhabitat selection. Grey shaded bars indicate the presence of experimental odour, white shaded bars indicate the presence of own odour, hatched bars delimitate the warmer terraria side, and unhatched bars the cooler terraria side. Means ± SE of the transformed amount of time are given for each terraria side. Significant individual contrasts between two terraria sides of the same habitat type x threat magnitude group are indicated: ** P = 0.005, * 0.01 < P < 0.05.

According to previous work (*e.g.* Rand 1964; Amo *et al.* 2004a; Amo *et al.* 2008), we predicted that *(a)* lizards should generally avoid threat risk. Our results are in line with these predictions, since in the *no trade-off* habitat lizards exposed to threats (experimental odour of males, females, and predators) avoided the experimental odour.

According to Van Damme *et al.* (1990a), we predicted that *(b)* lizards exposed to threat risk should prepare for potentially stressful situations and thus spend more time on the warmer terraria side. Again F, M, and P lizards of the *no trade-off* habitat spent more time on the warmer side, while C lizards selected cooler microhabitat in both habitat types. The fact that C lizards spent five times more time on the cooler compared to the warmer side, confirms that the common lizard is a highly temperature sensitive species that shows important behavioural differences with respect to temperature difference of 1.2° C. This indicates that the here found effects are a conservative estimate of behavioural responses to natural temperature differences, which are usually in the range of several degrees (Van Damme *et al.* 1990b). Since in lizards, time exposed to heat is positively correlated with T_b (Díaz *et al.* 1996), our results suggest that T_b of lizards spending more time on the warmer side was higher. Higher T_b increases sprint speed and stamina (Bennett 1983; Pinch and Claussen 2003), the first reaching a maximum at a T_b of 35° C in *L. vivipara* (Van Damme *et al.* 1990b). This indicates that lizards staying on the warmer side (M, F, and P lizards) may have selected warmer habitat in order to increase sprint speed and thereby their escape capabilities (Moberly 1968; John-Alder and Bennett 1981; Bennett 1983; Chen *et al.* 2003; Pinch and Claussen 2003). Our findings are thus in line with observations in *Lacerta monticola*, whose time spent in a refuge after a predator attack decreased with increasing T_b (Martín and López 1999; 2001). Similarly, *Anolis lineatopus* showed reduced flight distance with increasing T_b (Rand 1964), and *Klauberina riversiana* males thermoregulated closer to optimal T_b in the presence of competing males (Regal 1970). In contrast to F, M, and P lizards of the *no trade-off* habitat, C lizards selected cooler microhabitat. Since C lizards were not exposed to threat risk, they probably avoided warmer microhabitat in order to avoid high metabolic costs associated with higher T_b (Bennett 1983; Chen *et al.* 2003). The fact that C lizards spent more time on the cooler side irrespective of the presence of own odour shows that selecting microhabitat with optimal temperatures is more important than selecting familiar habitat.

According to the threat-sensitivity hypothesis (Helfman 1989) we predicted that *(c)* behavioural reactions should depend on the threat magnitude and that more acute behavioural reactions should be observed in lizards exposed to predator odour than in those exposed to conspecific odour and C lizards. Our results are in line with these predictions, since, compared to F, M, and C lizards, lizards exposed to predator odour showed more exploratory behaviour and appeasement displays. No differences existed in theses variables between F, M, and C lizards. This indicates that exploratory and appeasement behaviour were a specific response towards predators (Thoen *et al.* 1986; Van Damme *et al.* 1990a) and that both behaviours are of reduced importance in the presence of conspecific odour and in the control situation. Compared to the other groups, P lizards of *trade-off* habitat increased the amount of time moving. This suggests that P lizards of *trade-off* habitat may have increased shuttling movements between more or less suitable microhabitats for thermoregulation and between microhabitats with higher or lower predation risk, in order to reduce predation risk (Huey and Slatkin 1976). These results are in line with a gradual response depending on threat magnitude as predicted by the threat-sensitivity hypothesis (Helfman 1989). They show that exploratory and appeasement displays were only increased in the presence of high magnitudes of threat (presence of predator) and that more costly behaviours such as increased moving were only adopted in the presence of the highest threat magnitude (predator presence in the *trade-off* habitat), where warmer microhabitat could only be selected in the presence of high threat risk.

To test whether intrinsic habitat characteristics may affect the above-described reactions towards predators and conspecifics, we submitted lizards to a *no trade-off* and to a *trade-off* situation. In the no trade-off situation lizards could avoid the experimental odour and select warmer microhabitat. A second group faced a *trade-off* situation in which they could either select the warmer microhabitat at the expense of threat risk, or could avoid threat risk but consequently could not select the warmer microhabitat. As predicted (prediction *(d)* and *(e)*), F, M, and P lizards of the *trade-off* habitat spent less time on the warmer side than F, M, and P lizards of the *no trade-off* habitat and tended to increase the time spent on the side with threat risk. This suggests that M, F, and P lizards of the *trade-off* habitat faced indeed a trade-off situation and tried to maximize both, selection of warmer habitat

and avoidance of habitat with threat risk. As a consequence they spent less time in the warmer microhabitat and more time in the microhabitat with threat risk than *no trade-off* lizards. These findings are in line with results of a predator-prey model (Mitchell & Angilletta 2009) and experimental evidence (Van Damme *et al.* 1990a), showing that prey should adjust microhabitat choice and thermoregulation depending on predation risk. More specifically, thermoregulatory behaviour should be reduced in situations where costs associated with thermoregulation (*e.g.* threat risk) do not outweigh the benefits (see Van Damme *et al.* 1990a; Huey 1974; Huey and Webster 1976) and thus in the *trade-off* habitat.

Surprisingly, the reaction towards predator odour in *trade-off* and *no trade-off* habitats did not differ from the reaction towards conspecific odour neither in terms of thermal preferences nor in terms of risk avoidance, suggesting that common lizards also prepare for potential intraspecific interactions. This is clearly in contrast to the long-standing belief that the common lizard is a non-territorial species with high social tolerance, and it is in line with observed density-dependent aggressiveness among adult males (Lecomte *et al.* 1994; Aragón *et al.* 2006a), with aggressive interactions between female *L. vivipara* for limited resources (Vercken and Clobert 2008), and with studies in other species (*e.g.* Bramley *et al.* 2000; Amo *et al.* 2004b). However, M, F and C lizards showed significantly different exploratory or appeasement behaviours compared to P lizards, suggesting that the perceived threat magnitude originating from conspecifics was lower, despite similar microhabitat preferences. Furthermore, no significant interactions between sex and threat magnitude existed in M and F lizards, indicating that conspecific avoidance does not depend on inter- and/or intra-sexual interactions. Together with previous findings (Aragón *et al.* 2006a; Vercken and Clobert 2008) this suggests that conspecific competition may be more important for life-history strategies than previously believed, even for social and non-territorial species.

The fact that microhabitat selection depended on the presence/absence of a trade-off situation, experimentally demonstrates that optimal behaviours with respect to threat risk and threat magnitude are affected by intrinsic habitat characteristics. This proofs the existence of multifactorial integration based on threat risk, threat magnitude, and intrinsic habitat characteristics. Behavioural reactions followed a hierarchical order from low cost to high cost

behaviours. In the absence of threat risk (the control situation), lizards preferred the cooler side of the terrarium and thus avoided costs associated with warmer thermal conditions. In the presence of conspecifics or predators odour (presence of threat risk), lizards stayed on the warmer side and avoided threat risk. They thus preferred paying the costs associated with warmer thermal conditions, in order to prepare for potential interactions. Threat avoidance and warmer microhabitat preference were similarly important since in the *trade-off* situation no differences existed in the amount of time spent conducting one of the two behaviours. In the presence of high threat magnitude, *i.e.* the presence of predator odour, lizards increased the amount of exploratory behaviour and appeasement displays, and in the *trade-off* habitat in the presence of predator odour (representing the highest threat magnitude), they increased locomotor activity. In the latter situation, they thus additionally expended energy for locomotion. This demonstrates the existence of a behavioural hierarchy from low-cost behaviours in situations with low threat magnitude (*i.e.* avoidance of warmer thermal conditions) to selection of warmer habitat, increased exploratory and appeasement behaviour, and finally to high-cost behaviours in the high threat magnitude situations (*i.e.* reduced preference for warmer microhabitats and increased locomotor activity). Lizards thus gradually adjusted their behavioural response with respect to threat risk, threat magnitude, and intrinsic habitat characteristics.

In summary, our results show that microhabitat selection of common lizards follows a complex multifactorial integration of thermal conditions, threat risk, threat magnitude, and intrinsic habitat characteristics. Behavioural responses followed a behavioural hierarchy from low-cost behaviours associated with low threat magnitude situations to high-cost behaviours associated with high threat magnitude situations. Optimal behavioural solutions thus depend on multi-factorial integration including both biotic and abiotic factors, and they depend on interactions between threat risk, threat magnitude, and intrinsic habitat characteristics. Our results thus suggest that microhabitat selection is a complex task that depends on species-specific behavioural costs and complex interactions, potentially having several optimal solutions. The fine-tuned behavioural reactions towards conspecific odour displayed by this social and non-territorial species, suggests that multifactorial integration in habitat selection may be important in territorial and asocial as well as non-territorial

and social species. It may thus be more important than previously recognized and prevalent in a wide range of species ranging from unicellular organisms to insects and mammals.

Specific Acknowledgments

We thank Luis M. San-Jose, Elisa Pérez Badás and Jesús Ortega Giménez for field assistance. We also thank Pedro Aragón, Katleen Huyghe and Luis M. San-Jose for comments on the manuscript and Owen Howard and Elisa Hernández de Pablo for English correction. The capture and handling of lizards was conducted under the licenses provided by the Departamento de Medio Ambiente, Ordenación del Territorio y Vivienda (Gobierno de Navarra) and in agreement with the ABS/ASAB guidelines for ethical treatment of animals. VGJ was supported by a PhD grant from the Spanish Ministry of Education and Science (FPU AP2006-01678) and PSF by a grant from the Spanish Ministry of Education and Science (Programa Ramón y Cajal, RYC-2003-006136). The project was financed by the Spanish Ministry of Education and Science (CGL2005-01187, CGL2008-01522) and the Swiss National Science Foundation (PPOOP3_128375). The experiment described herein complies with the current laws of Spain.

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Chapter 5

5

Corticosterone effects on mate choice I: Blood corticosterone levels and intersexual selection games: best-of-bad-job strategies of male common lizards

Gonzalez-Jimena V, Fitze P (2012) *Behavioral Ecology and Sociobiology* 66: 305-315

Abstract

Glucocorticoids affect physiology and behaviour, reproduction and potentially sexual selection as well. Short-term and moderate glucocorticoid elevations are suggested to be adaptive, and prolonged and high elevations may be extremely harmful. This suggests that optimal reproductive strategies, and thus sexual selection, may be dose dependent. Here we investigate effects of moderate and high elevations of blood corticosterone levels on intra- and intersexual behaviour and mating success of male common lizards *Lacerta vivipara*. Females showed less interest and more aggressive behaviour towards high corticosterone males and blood corticosterone levels affected male reproductive strategy. Males of moderate and high corticosterone elevations, compared with Control males, showed increased interest (*i.e.* higher number of chases, tongue extrusions, and approaches) towards females and high corticosterone males initiated more copulation attempts. However, neither increased male interest nor increased copulation attempts resulted in more copulations. This provides evidence for a best-of-a-bad-job strategy, where males with higher corticosterone levels compensated for reduced female interest and increased aggressive female behaviour directed towards them, by showing higher interest and by conducting more copulation attempts. Blood corticosterone levels affected intrasexual selection as well since moderate corticosterone levels positively affected male dominance, but dominance did not affect mating success. These findings underline the importance of female mate choice and are in line with adaptive compensatory behaviours of males. They further show that glucocorticoid effects on behaviour are dose dependent and that they have important implications for sexual selection and social interactions, and might potentially affect Darwinian fitness.

Keywords:

Behavioural stress response ◆ Intersexual selection ◆ Intrasexual selection ◆ Lacertids ◆ Sexual signal

Efectos de la corticosterona sobre la selección de pareja I:
Niveles de corticosterona en sangre y selección intersexual: estrategias de los machos de lagartija de turbera

Resumen

Los glucocorticoides pueden afectar tanto a la fisiología como al comportamiento de los animales, incluido el comportamiento relacionado con la reproducción, y por tanto, pueden afectar potencialmente a la selección sexual. Se sabe que los efectos del aumento moderado en los niveles de corticosterona o del aumento producido durante un corto periodo de tiempo pueden ser adaptativos. Sin embargo, los efectos de un aumento elevado o de un aumento sostenido durante largos periodos de tiempo pueden llegar a ser extremadamente perjudiciales. Lo anterior sugiere que las estrategias reproductivas, y por tanto la selección sexual, pueden depender del nivel de corticosterona. En el presente experimento se investigaron los efectos de elevaciones moderadas y altas en los niveles de corticosterona en sangre sobre el comportamiento intra- e intersexual, y sobre el éxito de cópula de machos de lagartija de turbera (*Lacerta vivipara*). Se obtuvo que las hembras mostraban menos interés y mas comportamientos relacionados con el rechazo frente a machos con niveles más altos de corticosterona. Además, los niveles de corticosterona afectaron a la estrategia reproductiva de los machos, siendo los machos con niveles moderados y altos de corticosterona los que mostraban más interés hacia las hembras en comparación con machos control. Asimismo, los machos con niveles de corticosterona más elevados intentaron acoplarse con las hembras más veces que los demás. Sin embargo, ni el mayor interés en las hembras ni el aumento en los intentos de cópula de estos machos resultaron en un mayor número de cópulas. Los resultados obtenidos sugieren que los machos intentan sacar lo mejor de una mala situación desarrollando una estrategia de tipo “best-of-a-bad-job” por la cual los machos con mayores niveles de corticosterona intentan compensar el reducido interés y el rechazo de las hembras mostrando un mayor interés en ellas e intentando acoplarse más veces que los demás. Por otro lado, los niveles de corticosterona afectaron también a la selección intrasexual puesto que los machos con niveles moderados de corticosterona resultaron ser los más dominantes, aunque finalmente este estado de dominancia no afectó al éxito de cópula de los mismos. Los resultados obtenidos subrayan la importancia de la selección de pareja por parte de las hembras y además muestran que los efectos de la corticosterona en el comportamiento dependen del nivel que ésta alcance en sangre. Todo esto podría tener importantes implicaciones tanto para la selección

sexual como para las interacciones sociales, pudiendo afectar de manera potencial a la eficacia biológica de los individuos.

Palabras clave:

Lacértidos ◆ Respuesta de comportamiento al estrés ◆ Selección intersexual ◆ Selección intrasexual ◆ Señal sexual

Introduction

In the last years, the implications of stress and associated glucocorticoid hormone levels for animal behaviour and life history traits became a topic of major interest (see Roberts *et al.* 2007). In stressful situations, plasma glucocorticoid levels usually show a dramatic increase within few minutes (Selye 1936; Nelson 2005). It is suggested that short-term glucocorticoid elevations may be an adaptive response to stress, in order to optimise Darwinian fitness (Nelson 2005). Short-term glucocorticoid elevations suppress physiological functions that are not of fundamental importance at the moment of stress perception (*e.g.* immune system, digestion, reproductive processes, etc.), and they enhance functions critical for immediate survival (*e.g.* sensory functions and memory, Wingfield *et al.* 1998; Sapolsky *et al.* 2000; Pravosudov 2003; Berger *et al.* 2005). In contrast, prolonged elevations of glucocorticoid levels are believed to be extremely damaging (*e.g.* Selye 1936; McEwen and Sapolsky 1995; Morici *et al.* 1997). Glucocorticoids not only affect physiology but as well animal behaviour (Wingfield *et al.* 1998). Elevated glucocorticoid levels increase locomotor activity, change behavioural time budget (Belliure *et al.* 2004; Cote *et al.* 2006), decrease thermoregulatory activity (Belliure and Clobert 2004), influence dispersal behaviour (Vercken *et al.* 2007), and are suggested to inhibit intrasexual competition (Tokarz 1987; DeNardo and Licht 1993).

Several studies suggest that corticosterone (one of the most important stress related glucocorticoid hormones in reptiles, amphibians, birds, and many small mammals) affects reproduction and sexual selection (Evans *et al.* 2000; Sapolsky *et al.* 2000) and therefore Darwinian fitness. However, results of studies investigating effects on sexual selection are incongruent. For example, DeNardo and Licht (1993) did not find an effect of increased corticosterone levels on male sexual behaviour of side-blotched lizards (*Uta stansburiana*). This is in contrast to the general belief that an elevation of corticosterone levels inhibits reproductive behaviour (Sapolsky *et al.* 2000) and in zebra finches and Great Plain toads it has been shown that females prefer males with lower corticosterone levels (Leary *et al.* 2006; Roberts *et al.* 2007). Female preference for males with lower corticosterone levels may have evolved because of the detrimental effects of corticosterone (*e.g.* Oppliger *et al.* 1998; Sockman and Schwabl 2001; Wingfield and Kitaysky 2002; Berger *et al.* 2005),

potentially reducing male quality. However, high corticosterone levels may also decrease aggressive interactions between males (DeNardo and Licht 1993), and thus affect intrasexual selection that may be an important component of male mating success (Andersson 1994). This suggests that males with increased corticosterone levels may not be able to dominate rivals and thus may have reduced mating success. Crucial parameters for the effects of corticosterone on behavioural responses are the duration and magnitude of corticosterone elevation. Moderately elevated corticosterone levels are less deleterious for hippocampal anatomy and neurogenesis (Pravosudov and Omanska 2005) than high chronic elevations (Sapolsky 1996; McEwen 2000), they enhance spatial memory (Pravosudov 2003) and are observed when foraging conditions are most demanding (Silverin 1998), or when food supply is temporally limited or unpredictable (Pravosudov *et al.* 2001). This suggests that moderate elevations of corticosterone levels may produce different behavioural responses than severe elevations and that the effects of corticosterone on sexual selection may be dose dependent. However, despite clear theoretical predictions and incongruent results, few experimental studies have investigated the effects of corticosterone on intra- and intersexual selection and on male mating strategies (Tokarz 1987; DeNardo and Licht 1993; Hanley and Stamps 2002; Leary *et al.* 2006; Roberts *et al.* 2007) and studies investigating dose-dependent effects on sexual selection are lacking.

Here we investigated dose-dependent corticosterone effects (moderate and high corticosterone elevation) on intra and intersexual behaviour and mating success of male common lizards. We simultaneously presented females to three males of different corticosterone treatments (control, moderate, and high corticosterone levels) and registered inter and intrasexual behaviour during 3 hours. We predicted reduced mating success of high corticosterone males due to reduced sexual attractiveness and reduced competitive capacity (DeNardo and Licht 1993). However, given that adaptive behavioural strategies may depend on corticosterone levels (Pravosudov 2003; Cote *et al.* 2006; Vercken *et al.* 2007), male reproductive strategies may be dose dependent, and alternative mating strategies such as best-of-bad-job strategies may be adopted. Best-of-bad-job strategies are usually adopted by individuals in suboptimal conditions (*e.g.*, increased corticosterone levels), in order to reduce the associated fitness costs (see Koprowski 1993;

Møller and Birkhead 1993; Kempenaers *et al.* 1995; Watson *et al.* 1998; Lee and Hays 2004). Therefore, animals that adopt best-of-bad-job strategies may not necessarily suffer from reduced reproductive success, but they inevitably suffer from increased costs of mating (*e.g.* time and energy devoted to mating, energetic costs of reproductive behaviours, increased risk of predation, etc.; Watson *et al.* 1998; Lee and Hays 2004). Under this hypothesis, we predicted that corticosterone-treated males may compensate reduced attractiveness by adopting alternative mating strategies, and thus suffer from increased costs of mating. We as well predicted the existence of dose-dependent effects of corticosterone elevation on male behavioural strategies. More specifically, we predicted that moderate corticosterone elevations, due to their suggested adaptive role, might positively affect male mating success (see Pravosudov 2003) while high elevations may impair male mating success (Sapolsky *et al.* 2000; Leary *et al.* 2006; Roberts *et al.* 2007).

Specific Material and Methods

Pre-experimental conditions

All animals used for this experiment originated from one population at Somport (Huesca). During spring the population was regularly inspected to register male and female emergence from hibernation. Mating activity was determined by the presence of mating scars on the female's belly (Bauwens and Verheyen 1985). The first female with mating scars was detected on May 4th and immediately after detection we started capturing lizards (48 adult males and 16 adult females). Lizards were fed every four days with moth larvae (*Galleria mellonella*, Pyralidae). Lizards were randomly distributed among shelves and floors. No significant effects of shelf, floor, or their interaction on treatment group, sex, SVL, body mass, and body condition existed ($P > 0.5$).

Corticosterone treatment organization

In order to ensure that all individuals could be treated under the same standardized conditions we made 3 blocks of 18 (B1), 15 (B2) and 15 males (B3) each consisting of equal numbers of individuals per treatment group. There were no significant differences between blocks in SVL ($F_{2,47}$ = 0.074, P = 0.930), body mass ($F_{2,47}$ = 0.084, P = 0.919), or body condition ($F_{2,47}$ = 0.579, P = 0.565). Corticosterone application started on May 9th for B1 males, on 16th for B2 males, and on May 18th

for B3 males. In the morning and in the afternoon we applied during two subsequent days a dose of the assigned treatment on the lizard's back (for more details see Meylan *et al.* 2003 and Chapter 3). The following morning we started the mating trials. During the mating trials one dose of the assigned treatment was applied in the afternoon, while the morning application was suspended to make sure that treatment application did not disturb the behavioural assays.

Experimental design

Within blocks males were attributed to a male triad and within triads males were of similar SVL, body mass and body condition, but differed in corticosterone treatment. Each male triad was then assigned to a female. There were no significant differences between blocks in female SVL ($F_{2,47}$ = 1.242, P = 0.299), body mass ($F_{2,47}$ = 0.805, P = 0.453), or body condition ($F_{2,47}$ = 1.389, P = 0.260).

The mating experiment lasted from May 11th to May 21st 2008 (B1: May 11th and 12th, B2: May 18th and 19th, and B3: May 20th and 21st). At the start of the mating trials, we introduced a randomly chosen female into an escape-proof wooden box (2500 cm^2) and thereafter we introduced the three males of one triad simultaneously. Each male was marked for individual recognition. Males and females could interact for 3 h and were thereafter placed back into the individual terraria. If lizards were still copulating after 3h, we removed them 5 minutes after the end of the copulation. For B1 three simultaneous mating trials started at 09:00 hours and thereafter another three mating trials were conducted. For B2, two simultaneous mating trials started at 09:00 hours and thereafter another three were conducted, and for B3 three simultaneous mating trials started at 09:00 hours and thereafter another two were conducted. The last daily experiment started no later than at 13:30 hours.

Behavioural variables

Male behaviour was quantified once all lizards were released in the mating arena. We defined "actor" as the acting male and "receiver" (male or female) as the targeted lizard (*e.g.* if male no. 3 bites male no. 2 then no. 3 is the actor and no. 2 is the receiver). Each actor could interact with two receiver males of a different corticosterone treatment and with the female. If more than two lizards were interacting, we registered the behaviour of all involved lizard (*e.g.* male no. 1 approaches males no. 2 and 3, and male no. 2 is

simultaneously biting male no. 3. Here, we registered an approach of male no. 1 to 2, an approach of male no. 1 to, and a bite conducted by male no. 2 towards male no. 3). For each male we recorded or calculated the following variables that reflect mating behaviours:

– **1**. Probability of copulations: we registered it as 1 if a male gripped the female with the mouth on the posterior abdomen, successfully twisted his body around her, and copulated with her (*e.g.* Bauwens and Verheyen 1985; Fitze *et al.* 2010), or 0 if the male did not copulate with her.

– **2**. Number of pre-copulations: number of times a male gripped the female with the mouth on her posterior abdomen and tried to twist his body around her in order to copulate. Not all pre-copulations end with copulation because females are able to resist copulation attempts (Fitze *et al.* 2005; Fitze *et al.* 2010).

– **3**. Average copulation duration: average duration of the copula (in seconds). In some reptile species, copula duration has been shown to be positively correlated with sperm ejaculate volume and it has been suggested to be an indicator of male quality (Olsson 2001). None of the observed copulas ended due to disturbance by one of the not copulating males.

– **4**. Average pre-copula duration: average duration (in seconds) measured over all registered pre-copulations. Pre-copula duration is a measure of male interest.

For each male, we as well recorded the following variables, reflecting intersexual, if conducted towards females, and intrasexual interactions, if conducted towards males:

– **1**. Number of tongue extrusions: number of times a male rapidly extruded its tongue towards another lizard. Tongue flicking rate is a measure of exploratory activity (Halpern 1992) and reflects the strength of a response to a stimulus (Cooper Jr. and Burghardt 1990).

– **2**. Number of approaches: number of times a lizard approached another lizard or passed by at a close distance and simultaneously looked at it. Approaching another lizard is an indicator of interest and of potential interaction with the receiver (see, for example, López and Martín 2002).

– **3**. Number of chases: number of times a lizard persecuted another lizard. The majority of chases ended up in fighting with or in

biting the receiver (see Fitze *et al.* 2007).

– **4**. Number of Bites: number of times a lizard bites or tries to bite another lizard. The number of bites is an indicator of the level of aggressiveness (Vercken and Clobert 2008).

– **5**. Average bite duration: we also recorded the duration of each bite and calculated the average bite duration. Bites last from a fraction of a second up to several minutes (mean ± SE = 25.924 ± 10.729 s; minimum = 0 s; and maximum = 1,396 s) and were quantified with a precision of 1 s. Average bite duration does not include pre-copulatory or copulatory bites. Bite strength is linked to dominance (Huyghe *et al.* 2005), suggesting that bite duration may also be related to dominance, but clear evidence is lacking.

– **6**. Number of escapes: number of times a lizard tries to escape from another lizard by accelerating its movement explosively. Importantly, if an actor attacked a receiver, the receiver was fleeing, and the actor persecuted him, the behaviour was attributed to chasing (actor chases receiver). The number of escapes has been suggested to reflect a lizard's avoidance strategy and thus should be interpreted as a submissive behaviour (Vercken and Clobert 2008).

– **7**. Number of appeasement displays: number of times a lizard performs a series of rapid side-to-side movements with the tail or the tail tip in front of another lizard. This measure also includes the number of times a lizard performs a series of rapid up-and-down movements with its forelimbs (Thoen *et al.* 1986) in front of another lizard. Both behaviours are usually exhibited during social interactions and are appeasement displays for avoiding costly interactions (Punzo 2007).

For females, we recorded the number of approaches conducted towards a specific male as a proxy of female interest and the number of times she was biting a specific male as a proxy of female aggressiveness against males, which may indicate female resistance. The latter behaviour was only observed following male copulation attempts.

Statistics

Analyses were run in R 2.11.1 (Free Software Foundation, GNU Project, Boston, MA, USA) and in JMP IN 4.0.3 (SAS Institute). Model selection started with the full model that included all parameters and their interactions and the final model was determined using backward elimination. For all tests the significance level was set at an α of 0.05 (two-tailed test). The

assumptions of the applied models were tested and if they were not met, transformations were applied.

To test for the existence of treatment effects on female behaviour towards males and on mating behaviour, we applied generalised linear mixed models via PQL with binomial or Poisson error distribution for binomial and count data respectively (Quinn and Keough 2002). We included triad as random effect, actor treatment as fixed effect, actor male SVL as a covariate, and the interaction between treatment and actor male SVL into the model. Because the variables describing intra- and intersexual interactions performed by actor males were not statistically independent (*i.e.* significant correlations existed: $r > 0.035$, $F_{1, 142} > 5.207$, $P < 0.024$), we first performed principal component analysis to summarise the behavioural data from intra- and intersexual interactions into independent parameters. To test for the existence of treatment effects on intra- and intersexual interactions, we applied linear mixed models using principal components as dependent variables. First, we tested whether corticosterone treatment affected male behaviour using mixed model ANCOVAs. For these analyses, we included triad as random effect, actor treatment as fixed effect, actor male SVL as a covariate and the interaction between treatment and actor male SVL into the model. Second, we tested whether actors conducted different amounts of behaviours towards different receiver categories using mixed model ANCOVAs. We used independent analyses for each actor treatment group and included triad as random effect, receiver category as fixed factor, actor male SVL as covariate and the interaction between actor male SVL and receiver category into the model.

Block was included as random factor in all applied models. Since no significant differences between blocks existed for any of the analyses (all $P > 0.1$) we removed block from the models. To localise significant differences between treatment groups we applied post hoc contrasts (Quinn and Keough 2002) and where necessary applied sequential Bonferroni corrections (Benjamini and Hochberg 1995) to adjust α for the increased probability of obtaining statistical significance from multiple testing.

Results

Female behaviour towards males

Female behaviour significantly depended on male treatment ($F_{2, 28} = 6.060$, $P = 0.006$; Control, 2.187

± 1.166 SE; Cort0.15, 2.062 ± 0.933 SE; Cort1.5, 0.812 ± 0.356 SE) but not on male SVL ($F_{1, 28}$ = 0.492, P = 0.489). Females performed significantly less approaches towards males with higher corticosterone levels than towards control males ($F_{1, 28}$ = 12.063, P_{adj} = 0.006) and males with moderately elevated corticosterone levels ($F_{1, 28}$ = 5.847, P_{adj} = 0.044). No significant differences existed between control males and males with moderately elevated corticosterone levels (Control vs. Cort0.15, $F_{1, 28}$ = 0.641, P_{adj} = 0.430). Female aggressiveness towards a male was, though not statistically significant, higher towards males with higher corticosterone levels than towards control males ($F_{2, 28}$ = 3.150, P = 0.058; Control, 0.875 ± 0.239 SE; Cort0.15, 1.937 ± 0.782 SE; Cort1.5, 2.437 ± 0.677 SE; individual contrasts, Control vs. Cort1.5, $F_{1, 28}$ = 5.247, P_{adj} = 0.089; Control vs. Cort0.15: $F_{1, 28}$ = 0.673, P_{adj} = 0.419). Female aggressiveness towards males did not depend on male SVL ($F_{1, 28}$ = 0.317, P = 0.578).

Mating behaviour

Actor treatment, actor male SVL, and their interaction significantly affected the number of pre-copulations (Table 5. 1). In Control and Cort0.15 actors the number of pre-copulations increased with SVL (Table 5. 1, Figure 5. 1), while in Cort1.5 males the number of pre-copulations was independent of SVL (Table 5. 1, Figure 5. 1). Cort1.5 males of small SVL initiated more pre-copulations, than males of the other two treatments, while the contrary was the case for bigger males (Figure 5. 1).

Actor treatment, actor male SVL, and their interaction did not

Table 5. 1. Effects of actor corticosterone treatment and actor male SVL on intersexual behaviour.

Dependent variable	Parameter	*F* (*P*)	% v exp
Probability of copulation	treatment	$F_{2,29}$ = 1.345 (0.276)	-
	SVL	$F_{1,29}$ = 0.268 (0.608)	-
N pre-copulations	**treatment**	**$F_{2,27}$ = 3.411 (0.048)**	**140**
	SVL	**$F_{1,27}$ = 4.969 (0.034)**	**102**
	treatment x SVL	**$F_{2,27}$ = 3.818 (0.035)**	**157**
Average copulation duration	treatment	$F_{2,10}$ = 2.468 (0.135)	-
	SVL	$F_{1,10}$ = 0.003 (0.960)	-
Average pre-copulation duration	treatment	$F_{2,14}$ = 0.371 (0.700)	-
	SVL	$F_{1,14}$ = 0.012 (0.914)	-

Shown are the results of the minimal adequate model including test statistics, *P* values, and percentage of variance explained (% v exp). Significant parameters are set in bold.

significantly affect the probability of copulation, average copulation duration, and average pre-copulation duration (Table 5. 1).

Intra- and intersexual interactions

Of the variance in intra- and intersexual interactions, 72.2% was explained by three principal components, all of which had eigenvalues greater than 1 (eigenvalues, PC1 = 2.380, PC2 = 1.426, and PC3 = 1.250). The first principal component accounted for 34.0% of the variance in behaviour. It described male aggressiveness since the two biting related behaviours loaded highest (Table 5. 2). The second principal component explained 20.4% of the variance and described submissive behaviour since the number of appeasement displays and escapes loaded highest (Table 5. 2). Finally, the third axis explained 17.9% of the variance, and the number of chases, tongue extrusions and approaches load highest (Table 5. 2). Thus, PC3 reflected interest in interacting with others. Bigger values indicate more aggressiveness, more submissive behaviour, and more interest given that the component loadings of the principal behavioural variables were positive.

Male aggressiveness (PC1)

Treatment did not significantly affect male aggressive behaviour ($F_{2, 29}$ = 0.693, P = 0.508) and actor male SVL ($F_{2, 29}$ = 0.326, P = 0.753), and its interaction with treatment were not significant ($F_{2, 27}$ = 0.845, P = 0.441). Further, male aggressive behaviour conducted towards different receiver categories was not significantly different for any of the actor treatment groups (Table 5. 3). In none of the analyses did body size affect male aggressiveness (Table 5. 3).

Table 5. 2. Principal components analysis of intra- and intersexual interactions.

Shown are the component loadings for three rotated components representing 72.2% of the total variance. Plotted in italics are the main explanatory variables.

	Components loadings		
Behaviours	PC1	PC2	PC3
N tongue extrusions	0,020	0,046	*0,785*
N approaches	0,263	0,225	*0,661*
N escapes	0,063	*0,823*	0,049
N chases	0,092	-0,085	*0,819*
N appeasement displays	-0,034	*0,834*	0,043
N bites	*0,923*	0,064	0,204
Average bite duration	*0,953*	-0,030	0,069

Submissive behaviour (PC2)

Actor treatment did not significantly affect submissive behaviour of males ($F_{2, 29}$ = 1.080, P = 0.353) and actor male SVL ($F_{1, 29}$ = 3.572, P = 0.070), and the interaction were not significant ($F_{2, 27}$ = 0.161, P = 0.852). However, there were significant differences in the submissive behaviour conducted by males towards receiver categories (Table 5. 3). Control males conducted significantly less submissive behaviours towards females than towards Cort0.15 males (female vs. Cort0.15, $F_{1, 29}$ = 9.134, P = 0.015; Figure 5. 2a), showed, though not statistically significant, less submissive behaviours towards females compared with receiver Cort1.5 males (female vs. Cort1.5, $F_{1, 29}$ = 4.574, P = 0.081; Figure 5. 2a), and did not discriminate between Cort1.5 and Cort0.15 males (Cort1.5 vs. Cort0.15, $F_{1, 29}$ = 0.780, P = 0.384; Figure 5. 2a). Cort1.5 males conducted significantly less submissive behaviours towards females than towards Cort0.15 males (female vs. Cort0.15, $F_{1, 29}$ = 11.652, P = 0.006; Figure 5. 2a), showed, though not statistically significant, less submissive behaviours towards control males compared with Cort0.15 males (Control vs. Cort0.15, $F_{1, 29}$ = 5.207, P = 0.060; Figure 5. 2a), and did not discriminate between Control males and females (Control vs. females, $F_{1, 29}$=1.281, P = 0.267; Figure 5. 2a). Cort0.15 males did not conduct significantly different amounts of submissive behaviour towards different receiver categories. Submissive behaviour directed towards different receiver categories increased, though not statistically significant, with actor male SVL in Cort0.15 males (Table 5. 3).

Interest (PC3)

Actor treatment ($F_{2, 29}$ = 1.800, P = 0.183), actor male SVL ($F_{1, 29}$ = 0.379, P = 0.547) and their interaction ($F_{2, 27}$ = 2.324, P = 0.117) did not significantly affect male interest. There were however significant differences in male interest towards receiver categories (Table 5. 3). Cort0.15 males showed significantly more interest towards females than towards Control or Cort1.5 males (females vs. Control, $F_{1, 29}$ = 10.376, P = 0.006; females vs. Cort1.5, $F_{1,29}$ = 20.366, P < 0.001; Figure 5. 2b) and did not discriminate between Control and Cort1.5 males (Control vs. Cort1.5, $F_{1, 29}$ = 1.970, P = 0.171; Figure 5. 2b). Cort1.5 as well showed significantly more interest towards females than towards the other males (females vs. Control, $F_{1, 29}$ = 15.780, P = 0.001; females vs. Cort0.15, $F_{1, 29}$ = 16.781, P = 0.001; Figure 5. 2b) and did not discriminate between the other

males (Control vs. Cort0.15, $F_{1, 29}$ = 0.021, P = 0.887; Figure 5. 2b). In contrast, Control males did not show significant differences in interest between the female and males, and neither among males (Table 5. 3; Figure 5. 2b).

Finally, interest towards receivers significantly increased with actor male SVL in Cort0.15 males, but not in Cort1.5 and Control males (Table 5. 3; Figure 5. 3).

Discussion

Our results show that corticosterone treatment affected female behaviour, intra- and intersexual male behaviour and male reproductive strategy. Both, moderate and high corticosterone males, but not control males, increased their interest for females and smaller males of the high corticosterone treatment initiated pre-copulas more often than moderate corticosterone and control males. Increased interest and higher frequency of copulation trials should lead to higher mating success, if male–male competition principally determines access to mate partners (Olsson and Madsen 1995). However, in our study, despite the increased interest of corticosterone-treated males, no significant differences in male mating success existed between treatment groups. This suggests that intersexual selection is an important factor determining access to females and that female common lizards resist copulation attempts (Fitze *et al.* 2005; Fitze *et al.* 2010), both being in line with previous findings (Fitze *et al.* 2007). On the other hand, if females show reduced interest for males of increased corticosterone levels and preferentially mate with males of lower corticosterone levels (Leary *et al.* 2006; Robert *et al.* 2009), control and moderate corticosterone males should have higher mating success. Although we show that females showed reduced interest for males of increased corticosterone levels, this hypothesis is not supported by our results since no differences in male mating success were present. Alternatively, successful copulation may be the result of a combination of female and male interests, as suggested for many species (Smuts and Smuts 1993; Reynolds 1996; Birkhead and Møller 1998; Jennions and Petrie 2000). Reduced female interest for low-quality males (corticosterone-treated males), may be compensated by those males by showing increased interest for females, and on the other hand, high interest of females for highquality males (Control males) may not oblige high-quality males to show high interest for females in order to successfully copulate. Our results show that females indeed

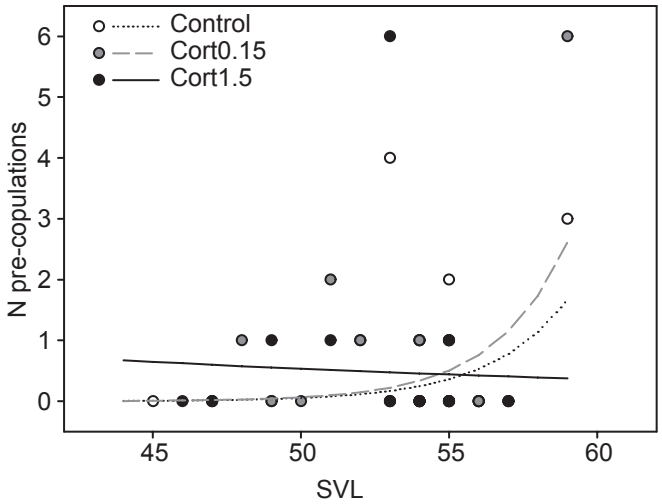


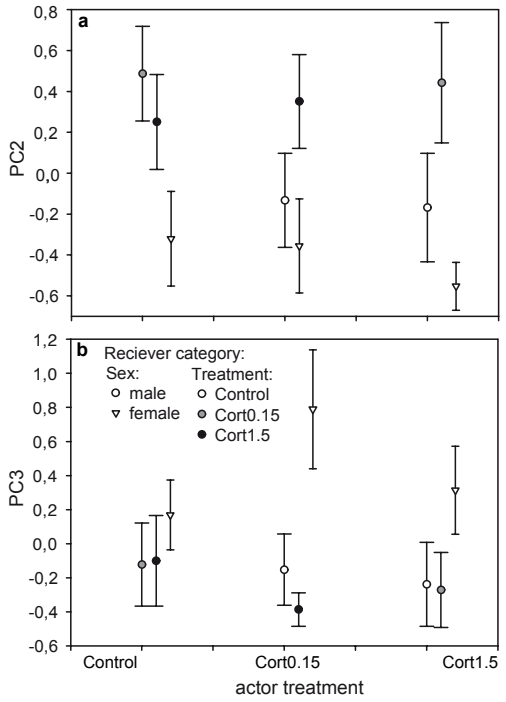
Figure 5. 1. Effects of actor treatment and SVL on the number of precopulations. Different shadings represent different actor treatment groups (Control (empty), Cort0.15 (grey), Cort1.5 (black)) and lines (black dotted for Control, grey dashed for Cort0.15, and black continuous for Cort1.5) correspond to the predicted relationships between actor male SVL and the number of pre-copulations for each actor treatment group.

preferred males with lower corticosterone levels and that they showed more aggressiveness against non-preferred males. These results are in line with the latter hypothesis and indicate that increased interest for females of corticosterone-treated males and the higher number of precopulations performed by smaller high corticosterone males may reflect a best-of-a-bad-job strategy (Koprowski 1993; Møller and Birkhead 1993; Kempenaers *et al.* 1995; Watson *et al.* 1998; Lee and Hays 2004) in order to cope with reduced female interest and increased female aggressiveness. Adopting a best-of-a-bad-job strategy led to no differences in mating success but significantly increased the energetic costs of mating, which is similar to findings by Lee and Hays (2004) where marine turtles suffered from male-induced injuries, but did not show differences in current reproductive success.

The increase in the number of pre-copulations with increasing body size in Control and moderate corticosterone, but not high corticosterone males, also suggests that the trade-off between current and future investment may have been directly affected by corticosterone treatment. Smaller Control and moderate corticosterone males may not have tried to copulate

because their investment may not have been rewarding enough, while all high corticosterone males tried to increase the mating probability, maybe due to reduced future prospects (*i.e.* reduced survival due to high corticosterone levels; Selye 1936; Morici *et al.* 1997). This finding is again in line with a best-of-a-bad-job strategy in order to increase mating success and it is similar to the observations by Kempenaers *et al.* (1995) in blue tits, where paternity is largely under female control and where unattractive (low-quality males), but not attractive males, perform mate guarding to reduce the probability of losing paternity.

Our results further show that Control and high corticosterone males were significantly more submissive towards moderate corticosterone males, indicating that moderate corticosterone males were dominant over the other males. Submissive behaviour of moderate corticosterone males did not differ between receiver categories which is in line with dominance of moderate corticosterone males. Although high corticosterone males were less preferred by females, they showed few submissive behaviours towards females, and significantly more submissive behaviour towards dominant males with moderate corticosterone elevation (Cort0.15).



towards different receivers by a particular actor treatment group. Means ±SE are given for receiver category, per actor treatment group. Actor treatment is represented on the x-axis. Symbols represent different sexes (males (dots) and females (triangles)) and shadings represent receiver treatment (Control (empty), Cort0.15 (grey), and Cort1.5 (black)).

Control and Cort1.5 males performed less submissive behaviour towards females than towards the other males, supporting the idea that during the breeding season intersexual interactions are potentially less harmful than intrasexual interactions due to male–male competition (Heulin 1988; Gvozdik and Van Damme 2003; Richard *et al.* 2005; Fitze *et al.* 2007), which could be the result of higher male

Figure 5. 2. Submissive behaviour (PC2) (a) and interest (PC3) (b) expressed

Table 5. 3. Differences in the aggressive behaviour (PC1), submissive behaviour (PC2), and interest (PC3) expressed by an actor of a particular treatment group towards different receiver categories.

		PC1		PC2		PC3	
Actor treatment	**Parameter**	*F* (*P*)	**%v exp**	*F* (*P*)	**%v exp**	*F* (*P*)	**%v exp**
Control	Receiver category	$F_{2,29}$ = 0.994 (0.396)	**-**	**$F_{2,29}$ = 4.830 (0.045)**	**15.5**	$F_{2,29}$ = 1.784 (0.279)	**-**
	SVL	$F_{1,29}$ = 1.293 (0.396)	-	$F_{1,29}$ = 2.366 (0.135)	-	$F_{1,29}$ = 0.013 (0.910)	-
Cort0,15	Receiver category	$F_{2,29}$ = 0.957 (0.396)	-	$F_{2,29}$ = 3.219 (0.082)	-	**$F_{2,29}$ = 10.577 (<0.001)**	**26.8**
	SVL	$F_{1,29}$ = 0.756 (0.396)	-	*$F_{1,29}$ = 4.534 (0.082)*	-	**$F_{1,29}$ = 12.353 (0.002)**	**15.7**
Cort1.5	Receiver category	$F_{2,29}$ = 1.548 (0.396)	-	**$F_{2,29}$ = 6.047 (0.036)**	**20.6**	**$F_{2,29}$ = 10.163 (<0.001)**	**17.1**
	SVL	$F_{1,29}$ = 0.971 (0.396)	-	$F_{1,29}$ = 2.410 (0.135)	-	$F_{1,29}$ = 0.981 (0.396)	-

Shown are test statistics, P values, and percentage of variance explained (% v exp). Significant parameters are plotted in bold and individual contrasts that were significant before adjusting α are italicised

than female bite force (Huyghe *et al.* 2005). Higher costs of male–male interactions might as well explain why Control and high corticosterone males performed more submissive behaviours towards dominant Cort0.15 males than towards other males. Our results also show that in males with moderate corticosterone levels, but not in the other males, the interest in interacting with other lizards increased with body size. Since body size is an important determinant of a lizard's dominance status (López and Martín 2001; Aragón *et al.* 2006) that as well determines fighting ability and a males' ability to harm (Gvozdik and Van Damme 2003; Huyghe *et al.* 2005), our results suggest that dominance affects body size-dependent behaviour since only dominant individuals (Cort0.15 lizards) expressed size-dependent interest (Table 5. 3), while in both Cort1.5 and control lizards no body size-dependent behaviour was apparent.

Previous experiments testing the effect of corticosterone on intrasexual male aggressiveness (Tokarz 1987; DeNardo and Licht 1993) showed that corticosterone reduces male aggression. Contrary to these studies, in our experiment actor corticosterone treatment or receiver category did not affect intra- and intersexual male aggression. These differences may be the consequence of different social contexts. In previous experiments, two males were interacting in the absence of a female (Tokarz 1987; DeNardo and Licht 1993; Hanley and Stamps 2002) and thus corticosterone-treated males may not have been motivated to compete. In contrast, we studied behavioural interactions of three males and one female. In this situation, intra- and intersexual selection could take place simultaneously, males were certainly motivated to compete for access to females, and male competition was stronger since three males had to compete for access to females. Nevertheless no significant effects of corticosterone on aggressive male behaviour were found. However, male dominance was corticosterone related and more submissive behaviour was conducted towards the dominant male than towards the other lizards. This indicates that the common lizard (as other lizard species, see Punzo 2007) generally avoids potentially aggressive interactions using subtle behavioural strategies (Gonzalez-Jimena and Fitze in preparation), *i.e.* submissive behaviour instead of aggression. This finding may as well explain why previous studies suggested that the common lizard has high social tolerance (see Cote and Clobert 2007a; Cote and Clobert 2007b; Cote *et al.* 2008; Vercken and Clobert 2008).

To our knowledge, this is the first study that simultaneously analyses intrasexual interactions among males of different corticosterone levels in an intersexual context. Our results support previous findings showing that females prefer males with low corticosterone levels, that reproductive behaviour is not inhibited by increased corticosterone levels (DeNardo and Licht 1993), and that moderate and high corticosterone levels induce shifts in male behaviour. In contrast to previous studies, we show that moderate elevations led to more dominant males and that both, males with high and moderate elevations, showed more interest for females but were not more successful in copulating than control males. This shows that intersexual selection through female mate choice importantly determines male mating success of common lizards (Fitze *et al.* 2005; Fitze *et al.* 2007; Fitze *et al.* 2010) and that corticosterone-treated males apply a best-of-a-bad-job strategy, probably in order to overcome female preferences for low corticosterone males. More generally, our results provide evidence for dose-dependent effects of corticosterone and they are in line with previous studies suggesting that moderate corticosterone elevations may be rather positive, and they indicate that both, moderate and high corticosterone elevations lead to potentially adaptive compensatory behaviours.

Specific Acknowledgments

We thank Luis M. San-Jose, Miguel Peñalver Alcázar and Sergio Cánovas Fajardo for field and laboratory assistance and two anonymous referees for very helpful comments. The capture and handling of lizards was conducted under the licenses provided by the Instituto Aragonés de Gestión Ambiental (Gobierno de Aragón). VGJ was supported by a Ph.D. grant from the Spanish Ministry of Education and Science (FPU AP2006-01678) and PSF by a grant from the Spanish Ministry of Education and Science (Programa Ramón y Cajal, RYC-2003-006136). The project was financed by two grants from the Spanish Ministry of Education and Science (CGL2005-01187, CGL2008-01522) and the Swiss National Science Foundation (PPOOP3_128375).

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Chapter 6

6

Corticosterone effects on mate choice II: Dose dependent corticosterone effects on female context dependent intersexual behaviours in the common lizard (*Lacerta vivipara* Jacquin, 1787)

Gonzalez-Jimena V, Fitze P , *in preparation*

Abstract

Stress response and its associated glucocorticoids levels are known to affect physiology and behaviour. It has been suggested that optimal reproductive strategies, and thus sexual selection, may dependent on glucocorticoid levels. Here we tested whether intersexual behaviour and female mate choice are corticosterone dependent by sequentially presenting three males with different corticosterone levels (high, moderate and control) to a female common lizard (*Lacerta vivipara*). We also tested for context dependent female choice with respect to corticosterone levels. Corticosterone had dose dependent effects on both male and female intersexual behaviour, but did not affect proxies strictly related with fitness, such as copulation probability or copulation duration. Female interest and defensive behaviour depended on male corticosterone level, male presentation order and female mating status. Male corticosterone levels also affected male interest, but not male reproductive behaviour per se. This shows that male corticosterone levels affect inter-sexual behaviour, context dependent female mate choice, and male reproductive strategy, but not traits related more closely with fitness. These results thus show that male corticosterone levels play an important role in sexual selection, but that their effect on individual fitness may be small.

Keywords:

Behavioural stress response ◆ Compensatory strategies ◆ Context dependent female choice ◆ Lacertids ◆ Sexual selection

Efectos de la corticosterona sobre la selección de pareja II:
Efectos dependientes de dosis de la corticosterona sobre el comportamiento intersexual contexto-dependiente en hembras de lagartija de turbera (*Lacerta vivipara* Jacquin, 1787)

Resumen

Se sabe que la respuesta al estrés y el aumento en los niveles basales de glucocorticoides que ésta conlleva pueden afectar tanto a la fisiología como al comportamiento de los individuos. Se ha sugerido que las estrategias reproductoras óptimas, y por tanto la selección sexual, pondrían depender de los niveles de glucocorticoides. En el presente capítulo se investiga si el comportamiento intersexual y la selección de pareja por parte de las hembras son dependientes de los niveles de corticosterona. Para ello se presentaron tres machos con diferentes niveles de corticosterona (elevado, moderado y control) de manera secuencial a una hembra de lagartija de turbera (*Lacerta vivipara*). Asimismo, se investigó la influencia de los niveles de corticosterona en la selección de pareja dependiente de contexto de las hembras. Se obtuvo que los niveles de corticosterona tuvieron efectos dependientes de dosis tanto en el comportamiento intersexual de los machos como en el de las hembras pero no afectaron a variables directamente relacionadas con la eficacia biológica de los individuos, tales como la probabilidad de copula o la duración de la misma. El interés de las hembras y su comportamiento defensivo se vieron afectados por los niveles de corticosterona de los machos, por el orden de presentación de los mismos y por el estado de apareamiento de la hembra. Los niveles de corticosterona de los machos afectaron además a su interés por las hembras pero no a su comportamiento más estrictamente reproductor. Los resultados muestran que los niveles de corticosterona de los machos afectan al comportamiento intersexual, a la selección de pareja dependiente de contexto por parte de las hembras y a la estrategia reproductora de los machos. Sin embargo, no muestran efectos directos sobre variables más directamente relacionadas con la eficacia biológica. Por tanto, se muestra que los niveles de corticosterona de los machos de lagartija de turbera pueden llegar a jugar un papel importante en la selección sexual, sin embargo su papel en la eficacia biológica a nivel individual parece ser pequeño.

Palabras clave:

Estrategias de compensación ◆ Lacértidos ◆ Respuesta conductual al estrés ◆ Selección de pareja dependiente de contexto ◆ Selección sexual

INTRODUCTION

In species where females gain direct benefits from choosing a male (*e.g.* through parental care, nuptial gifts) the existence of female mate choice is more obvious. However, in species where females do not gain direct benefits from choosing a given male, several hypotheses may explain why female mate choice exists (Andersson 1994; Jennions and Petrie 2000). One of the hypotheses for the existence of the latest is that female mate choice might enhance offspring's genetic diversity (Birkhead and Parker 1997) or quality by choosing good quality males (Reynolds and Gross 1992; Saino *et al.* 1997; Birkhead and Møller 1998; Kokko *et al.* 2003; Fitze *et al.* 2010). However, not always better quality males are chosen. In some species, it has been suggested that females first mate with a male to ensure fertilization, *i.e.* not necessarily with a good quality male, and thereafter become choosy and primarily mate with males of higher quality. Females thus trade-up males in order to maximize both reproductive success and offspring quality (Jennions and Petrie 2000; Pitcher *et al.* 2003). The existence of trade-up strategies as well implies that optimal female mate choice may depend on previous mating history and thus may be context dependent (*e.g.* Jia *et al.* 2000; Alonzo and Sinervo 2001; Qvarnstrom 2001; Kwiatkowski and Sullivan 2002; Pitcher *et al.* 2003; Hunt *et al.* 2005; Fitze *et al.* 2010). During the mating period females generally visit several males in a sequential way before mating (Bakker and Milinski 1991). Species where females simultaneously sample several potential mates in order to compare and select the best potential mates are quite rare (exceptions are for example lekking species, *e.g.* Kokko *et al.* 2003). Many hypothesis explaining how females compare and chose males have been developed (see Janetos and Cole 1981; Bakker and Milinski 1991; Pitcher *et al.* 2003) but context seems to play a principal role. Given the general importance of female strategies, little is known about male strategies, despite the fact that female strategies may reduce male individual fitness. In order to maximize fitness, males should develop compensatory strategies and behaviours for accessing females, *e.g.* sneaking strategy in *Uta stansburiana* (Sinervo and Lively 1996; Zamudio and Sinervo 2000) and *Lacerta vivipara* (Sinervo *et al.* 2007).

In the recent years, several studies suggest that stress and its related glucocorticoids hormones may have important implications for sexual selection and thus for mate choice (Evans *et al.* 2000; Sapolsky *et al.* 2000; Roberts ML *et al.* 2007).

Stress generally leads to a rapid increase in plasma glucocorticoids levels (Selye 1936; Nelson 2005). High levels of glucocorticoids usually suppress many physiological functions that are not important at the moment of stress perception, and enhance functions critical for immediate survival (Tokarz 1987; DeNardo and Licht 1993; Wingfield *et al.* 1998; Sapolsky *et al.* 2000; Pravosudov 2003; Belliure *et al.* 2004; Berger *et al.* 2005; Cote *et al.* 2006). One of the most important physiological functions that is inhibited by high glucocorticoids elevations is reproduction (Evans *et al.* 2000; Sapolsky *et al.* 2000). In reptiles, the principal glucocorticoid involved in the stress response is corticosterone (Nelson 2005). Corticosterone effects on reproduction are not well understood, and several hypotheses try to explain its effects on it. It may directly affect reproduction through physiological processes (such as the inhibition of reproduction inducing hormones, Nelson 2005) or indirectly through behavioural changes (Nelson 2005). For example, high corticosterone levels decreases aggressive interactions between males (DeNardo and Licht 1993) which makes them less successful when competing with other males for access to females. Furthermore, corticosterone may also inhibit courtship behaviour (Moore and Miller 1984). In addition, corticosterone may negatively affect males mating chances (*i.e.* female mate choice) through its negative effects on males' quality dependent cues. For example, corticosterone is immunosuppressive (Oppliger *et al.* 1998; Wingfield and Kitaysky 2002; Berger *et al.* 2005) and thus, males with higher corticosterone levels may suffer from a weakened immune system that may imply higher parasitic loads or low disease resistance. This immunosuppression may be reflected in sexually selected traits used by females in order to assess male quality (see Hamilton and Zuk 1982; Buchanan 2000; Evans *et al.* 2000) and thus, females may reject those males. In addition, female lizards use body condition dependent cues in male selection (see Fitze *et al.* 2007) *and* corticosterone decreases body condition (Sockman and Schwabl 2001; Berger *et al.* 2005). Therefore, females may also reject those males with lower body condition. Moreover, not only the magnitude of the corticosterone increase, but as well the duration of the increase is a crucial parameter modulating corticosterone effects. Whereas short-term corticosterone elevations are considered to be adaptive, prolonged elevations of corticosterone levels are believed to be extremely damaging (Selye 1936; McEwen and Sapolsky 1995;

Morici *et al.* 1997; Wingfield *et al.* 1998). However, little is known about elevations of corticosterone levels that only moderately exceed baseline (Pravosudov 2003). The few studies that investigated the effects of moderate elevations contrast to the deleterious effects reported previously and suggest that moderate elevations may not be deleterious (Pravosudov and Omanska 2005) but adaptive (Pravosudov 2003). However, little evidence exists and their effect on reproduction and sexual selection has hardly been studied.

To our knowledge, experimental studies investigating dose-dependent effects of corticosterone on male reproductive success, female mate choice, and sexual selection in general, are still scarce or even absent (Leary *et al.* 2006; Roberts *et al.* 2007). Further, few experimental studies have been conducted to investigate the direct effects of different corticosterone levels in behavioural interactions and thus, in social relations between conspecifics (Moore and Miller 1984; Tokarz 1987; DeNardo and Licht 1993; Hanley and Stamps 2002). Here we investigated, using the common lizard as a model species, if intersexual behaviours and female mate choice are corticosterone dependent, by sequentially presenting to a female three males with different corticosterone levels (high, moderate and control). We predicted that female interest in males would decrease with increasing corticosterone levels of males, and that female resistance behaviour towards males would increase with increasing corticosterone levels. If female mate choice is context dependent we predicted that females change their mate preferences according to their previous mating history. Finally, we expected that males would perform compensatory strategies to circumvent female preferences.

Specific Material and Methods

Pre-experimental conditions

During spring, we regularly inspected the Somport population (see *Chapter 3*) to register male and female emergence. For each captured female, mating activity was determined by the presence of mating scars on the female's belly (Bauwens and Verheyen 1985). When the first female with mating scars was detected, we started capturing male and female lizards (4 May 2008). The captured lizards were maintained under standardized conditions (see *Chapter 3*).

To assure that all lizards could be handled under the same standardized conditions we attributed them to three blocks. The first block included 6 females and 18 males,

and each of the other two blocks included 5 females and 15 males. There were no significant differences between blocks in SVL (females: $F_{2,47}$ = 1.242, P = 0.299; males: $F_{2,47}$ = 0.074, P = 0.930), body weight (females: $F_{2,47}$ = 0.805, P = 0.453; males: $F_{2,47}$ = 0.084, P = 0.919), or body condition (females: $F_{2,47}$ = 1.389, P = 0.260; males: $F_{2,47}$ = 0.579, P = 0.565). Animals were randomly distributed in the laboratory and no significant effect of shelf, shelf level, or their interaction existed in male corticosterone treatment frequency, sex frequency, SVL, body weight, and body condition ($P > 0.5$).

Corticosterone treatment organization

To investigate the effect of moderate and high corticosterone elevations on intersexual behaviour, we assigned 16 males to the "Cort1.5" treatment, another 16 males to the "Cort0.15" treatment, and the remaining 16 males to the "Control" treatment (see *Chapter 3* for corticosterone treatments specifications). There were no significant differences between corticosterone treatment groups in snout-vent length, SVL ($F_{2,47}$ = 0.007, P = 0.993), body mass ($F_{2,47}$ = 0.101, P = 0.904), and body condition ($F_{2,47}$ = 0.239, P = 0.789). Corticosterone treatment was applied on lizards' back in the morning and in the afternoon, when the lights of the terraria were turned-off (for more details see Meylan *et al.* 2003). We followed this procedure during two subsequent days, and in the following morning we started the mating trials. During the mating trials the morning dose was suspended to make sure that treatment application did not disturb the behavioural assays. The consequences of the here-applied treatments on plasma corticosterone levels are described in *Chapter 3*.

Experimental design

On May 11th (first block), May 18th (second block), and May 20th (third block), we sequentially presented to each female three males, one of each corticosterone treatment. We presented males to the females in a sequential way in order to avoid the potential effect of male intra-sexual competition in female mate choice, and in order to investigate context dependent female mate choice. Females were first introduced into an escape-proof wooden mating arena (2500 cm^2) and thereafter a single male was introduced. Every male could interact with the female for one hour and was thereafter removed. If lizards were copulating when removal should have been performed, we waited until the end of the copulation and five minutes

later removed the male. All presented males were unknown to the female. Male and female behaviour were registered. Males presented to the same female were of similar SVL (± 1.3 mm), body mass (± 0.4 g), and body condition (± 0.4). No significant differences in SVL, body mass and body condition existed between males of a different presentation order (SVL: $F_{2,47}$ = 0.077, P = 0.926; body weight: $F_{2,47}$ = 0.404, P = 0.670; body condition: $F_{2,47}$ = 1.953, P = 0.154) and corticosterone treatments were equally distributed among presentation orders (*Pearson* χ^2_4 = 0.750, P = 0.945). Female mating status (0 = not yet mated; 1 = previously mated) changed during the experiment, and there were no significant differences in female mating status among males of different presentation orders (*Pearson* χ^2_2 = 5.101, P = 0.080). Mating trials started at 09:00 hours and the last trial started no later than 13:30 hours.

Behavioural variables

During the entire experiment we recorded female behaviour directed towards males by counting the number of times they performed the following behaviours:

- **1**. Number of tongue extrusions: Number of times a female rapidly extruded its tongue towards another lizard. The number of tongue flicking measures exploratory activity (Halpern 1992). It has also been used as a reflect of the strength of the response towards a given stimulus (Cooper, Jr. and Burghardt 1990).

- **2**. Number of approaches: Number of times a lizard approached another lizard or passed by at a close distance and simultaneously looked at it. Approaching to another lizard might be interpreted as an indicator of interest and of potential interacting with the approached one (López and Martín 2002).

- **3**. Number of bites: Number of times a female bites or tries to bite a male. The number of biting attempts is likely to be an indicator of level of aggressiveness (Vercken and Clobert 2008) and thus, may indicate female resistance to mate (Fitze *et al.* 2010).

- **4**. Number of escapes: Number of times a female tries to escape from a male that is not chasing her by accelerating its movement explosively. The number of escapes is likely to reflect the tendency of a female to avoid competitive interactions and thus should be interpreted as a submissive behaviour (Vercken and Clobert 2008).

- **5**. Number of tail vibrations: Number of times a female performs

a series of rapid side-to-side movements with the tail or the tail tip in front of a male. This behaviour usually exhibit during social interactions and it is thus an appeasement display for avoiding costly interactions (Punzo 2007).

- **6**. Number of forelimbs movements: Number of times a female performs a series of rapid up-and-down movements with its forelimbs in front of a male (Thoen *et al.* 1986). This behaviour is also usually exhibited during social interactions and it is appeasement display for avoiding costly interactions as well (Punzo 2007).

- **7**. Number of pre-copulations: A female-male encounter was defined as pre-copulation when the male was gripping the female with the mouth on the posterior abdomen and twisting his body around her preparing for copulation. Not all pre-copulations end with copulation because females are able to resist male's copulation attempts (Fitze *et al.* 2005; Fitze *et al.* 2010).

- **8**. Number of copulations: A female-male encounter was defined as copulation when the male gripped the female with the mouth on the posterior abdomen and when he successfully twisted his body around her and initiates copulation (Fitze *et al.* 2007).

We as well recorded the duration (in seconds) of the following behaviours:

- **1**. Average pre-copula duration: Average duration measured over all registered pre-copulations. Pre-copula duration is a measure of male interest in mating that female (Fitze *et al.* 2005; Fitze *et al.* 2010).

- **2**. Average copulation duration: Average duration of the copula. In some reptile species, copula duration is positively correlated with sperm ejaculate volume. Therefore, it has been suggested to be an indicator of male quality (Olsson 2001).

Finally, we recorded the number of approaches, bites, and chases (number of times a lizard persecuted another lizard) conducted by a male towards the female as a proxy of male interest towards the female.

Statistics

Analyses were conducted using R 2.9.0 (Free Software Foundation, GNU Project, Boston, MA, USA) and JMP 8.0.2 (SAS Institute Inc).

First, principal component analysis (PCA) was performed to summarize intersexual interactions. To test whether corticosterone treatment affected intersexual interactions, we applied linear mixed models with the obtained principal components as dependent variables. To test for differences in the duration of the different behaviours among male

corticosterone treatment groups we applied linear mixed models including all females that successfully pre-copulated or copulated. Finally, to test for corticosterone treatment effects on male interest conducted towards the female we ran generalized linear mixed models via PQL with Poisson error distribution (Quinn and Keough 2002). In these models we included female as random effect, male corticosterone treatment and female mating status as fixed effects, and male presentation order as a covariate. Female mating status (0 = not mated yet; 1 = mated previously) was included to investigate context dependent female behaviour, *i.e.* if they exhibited different mating strategies before and after the first mating.

Model selection started with the full model that included all parameters and their interactions, and the final model was determined using backward elimination. For all tests model assumptions were tested and if they were not meet transformations of the dependent variables were applied. Significance level was set at $P \leq 0.05$ (two-tailed test).

To localize significant differences between treatment groups we applied post-hoc contrasts (Quinn and Keough 2002) and when necessary applied sequential Bonferroni corrections (Benjamini and Hochberg 1995) to adjust α for the increased probability of obtaining statistical significance from multiple testing.

Table 6. 1: Principal components analysis of intersexual interactions conducted by females.

	Components loadings			
Behaviours	**PC1**	**PC2**	**PC3**	**PC4**
N tongue extrusions	-0,003	-0,160	0,043	*0,879*
N approaches	-0,249	0,245	-0,125	*0,713*
N escapes	-0,145	0,566	*0,730*	0,081
N tail vibrations	-0,182	*0,704*	0,595	0,050
N forelimb movements	0,019	*0,943*	-0,016	-0,010
N bites	0,125	-0,064	*0,919*	-0,117
N pre-copulations	*0,929*	-0,099	0,059	-0,111
N copulations	*0,942*	0,009	-0,053	-0,102

Shown are the component loadings for the four rotated components (84.63% of the total variance). Plotted in italics are the main explanatory variables

Results

Intersexual interactions conducted by the female

Intersexual behaviour conducted by females was summarized into four principal components with eigenvalues greater than 1 (eigenvalues: PC1 = 2.653, PC2 = 2.009, PC3 = 1.103, PC4 = 1.006), which explained the 84.63% of the variance. The first principal component accounted for 33.16% of the variance in behaviour (Table 6. 1) and reflected mating behaviour, since the number of pre-copulations and copulations loaded highest. The second principal component explained 25.12% of the variance and described appeasement behaviour, since the number of forelimb movements and tail vibrations loaded highest (Table 6. 1). The third principal component explained 13.79% of the variance, and described defensive behaviour, since the number of bites and escapes loaded highest (Table 6. 1). Finally, the fourth principal component (12.57% of the variance) reflected interest in the male, since the number of tongue extrusions and approaches loaded highest (Table 6. 1). The component loadings of the principal behavioural variables were positive (Table 6. 1), and thus bigger values indicate more mating behaviour, appeasement behaviour, defensive behaviour, and more interest in interacting with males.

Mating behaviour (PC1) and appeasement behaviour (PC2)

There were no significant differences in female mating (PC1) and appeasement behaviour (PC2) directed towards males of different corticosterone treatment. Mating status and male presentation order did not affect mating and appeasement behaviour (Table 6. 2) and no significant interactions existed (all $P > 0.05$).

Defensive behaviour (PC3)

Defensive behaviour significantly depended on male corticosterone treatment (Table 6. 2). Females performed a higher amount of defensive behaviours towards Cort0.15 males than towards Control males ($F_{1,28} = 9.337$, $P_{adj} = 0.015$, Figure 6. 1a), and tended to perform more defensive behaviour towards Cort0.15 males than towards Cort1.5 males ($F_{1,28} = 4.382$, $P_{adj} = 0.067$, Figure 6. 1a). No significant differences were found between defensive behaviour performed by females towards Control and Cort1.5 males ($F_{1,28} = 0.825$, $P_{adj} = 0.371$). Mating status and presentation order did not affect defensive behaviour.

Table 6. 2: Effects of corticosterone treatment, female mating status male presentation order (order), and their interactions on mating behaviour (PC1), appeasement display (PC2), defensive behaviour (PC3), female sexual interest (PC4) and duration of mating behaviours.

Dependent variable	Parameter	*F* (*P*)	%v exp
PC1	Cortico	$F_{2,28}$ = 0.155 (0.857)	-
	Mating status	$F_{1,28}$ = 2.866 (0.102)	-
	Order	$F_{1,28}$ = 1.279 (0.268)	-
PC2	Cortico	$F_{2,28}$ = 1.447 (0.252)	-
	Mating status	$F_{1,28}$ = 1.348 (0.255)	-
	Order	$F_{1,28}$ = 0.064 (0.802)	-
PC3	**Cortico**	**$F_{2,28}$ = 5,361 (0.011)**	25.384
	Mating status	$F_{1,28}$ = 0,164 (0.689)	-
	Order	$F_{1,28}$ = 3,350 (0.078)	-
PC4	Cortico	$F_{1,21}$ = 2.134 (0.143)	-
	Mating status	**$F_{2,21}$ = 6.131 (0.022)**	4.848
	Order	**$F_{2,21}$ = 12.624 (0.002)**	9.984
	Cortico x Order	**$F_{1,21}$ = 14.158 (<0.001)**	22.390
	Mating status x Order	**$F_{1,21}$ = 18.197 (<0.001)**	14.391
	Cortico x Mating status	**$F_{2,21}$ = 4.442 (0.025)**	7.026
	Cortico x Mating status x Order	**$F_{2,21}$ = 13.513 (<0.001)**	21.373
Average pre-cop duration			-
	Cortico	$F_{2,4}$ = 0.152 (0.863)	
	Mating status	**$F_{1,4}$ = 8.407 (0.044)**	62.065
	Order	$F_{1,4}$ = 0.834 (0.413)	-
Average copulation duration			-
	Cortico	$F_{2,3}$ = 4.335 (0.130)	
	Mating status	$F_{1,3}$ = 0.226 (0.667)	-
	Order	$F_{1,3}$ = 2.500 (0.212)	-

Shown are the results of the final model including test statistics, *P*-values, and percentage of variance explained (% v exp).

Female sexual interest (PC4)

Female interest in interacting with males was significantly affected by the interaction between male corticosterone treatment, female mating status and male presentation order (Table 6. 2, Figure 6. 2). Post-hoc tests showed that female interest towards Control males decreased more steeply with presentation order in not mated females than in mated females (mated *vs.* not mated: $F_{1,21}$ = 33.139, P_{adj} < 0.001, Figure 6. 2, contrast a-b), while no significant slope differences between mated and not mated females existed in female interest towards Cort0.15 males and Cort1.5 males (mated *vs.* not mated: Cort0.15 males: $F_{1,21}$ = 4.105, P_{adj} = 0.101, Cort1.5males: $F_{1,21}$ = 0.791, P_{adj} = 0.576, Figure 6. 2).

Moreover, for not mated females, post-hoc tests showed that interest in interacting with Control males decreased more steeply with presentation order than interest in interacting with Cort0.15 and Cort1.5 males (Control vs. Cort0.15: $F_{1,21}$ = 57.506, P_{adj} < 0.001; Control vs. Cort1.5: $F_{1,21}$ = 28.775, P_{adj} < 0.001, Figure 6. 2a, contrasts a-c and a-d, respectively). In not mated females, interest in interacting with Cort0.15 males significantly increased with male presentation order in relation to Cort1.5 males (individual contrast for not mated females: Cort0.15 vs. Cort1.5: $F_{1,21}$ = 12.015, P_{adj} = 0.005; Figure 6. 2a, contrast c-d). In already mated females the relationship between interest in interacting with a male and presentation order did not significantly differ between male corticosterone treatments (individual contrast for mated females: $F_{1,21}$ < 0.235 , P_{adj} > 0.810, Figure 6. 2b).

Duration of mating behaviours

Female mating status, but not corticosterone treatment and mating order, significantly affected pre-copulation duration (Table 6. 2). Pre-copulations of mated females lasted longer than those of not mated females (mean ± SE: mated, 73 ± 26.52; not mated, 23.833 ± 9.435 SE). Corticosterone treatment, female mating status, and male presentation order did not significantly affected copulation duration (Table 6. 2).

Intersexual interactions conducted by the male

Male approaching behaviour towards females was significantly affected by male corticosterone treatment (mean ± SE: Control, 3.062 ± 1.253; Cort0.15, 5.875 ± 1.760; Cort1.5, 2.562 ± 0.831; $F_{2,28}$ = 9.634, P < 0.001). Cort0.15 males performed more approaches towards females than Control ($F_{1,28}$ = 10.081, P = 0.009, Figure 6. 3) and Cort1.5 males ($F_{1,28}$ = 15.034, P = 0.009, Figure 6. 3). Male approaching behaviour towards females was not significantly affected by female mating status ($F_{1,28}$ = 2.292, P = 0.098) nor by male presentation order ($F_{1,28}$ = 0.189, P = 0.667).

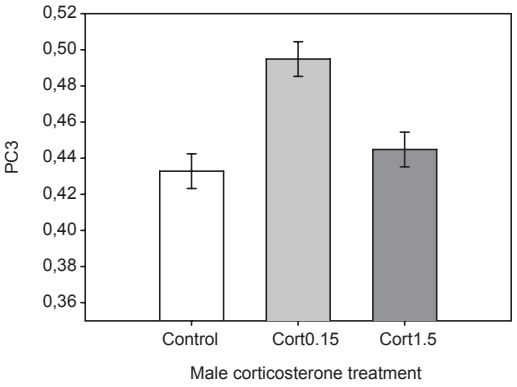

Figure 6. 1: Defensive behaviour (PC3*) of females towards males in relation to corticosterone treatment. Means ± SE are given and shading indicates the corticosterone treatment group (empty = Control, light grey = Cort0.15, dark grey = Cort1.5). * Ln transformed.

The number of times a male bit or chased the female were not significantly affected by male corticosterone treatment ($F_{2,28} \leq 1.524$, $P \geq 0.235$), female mating status ($F_{1,28} \leq 0.156$, $P \geq 0.705$), nor by male presentation order ($F_{1,28} \leq 0.365$, $P \geq 0.550$).

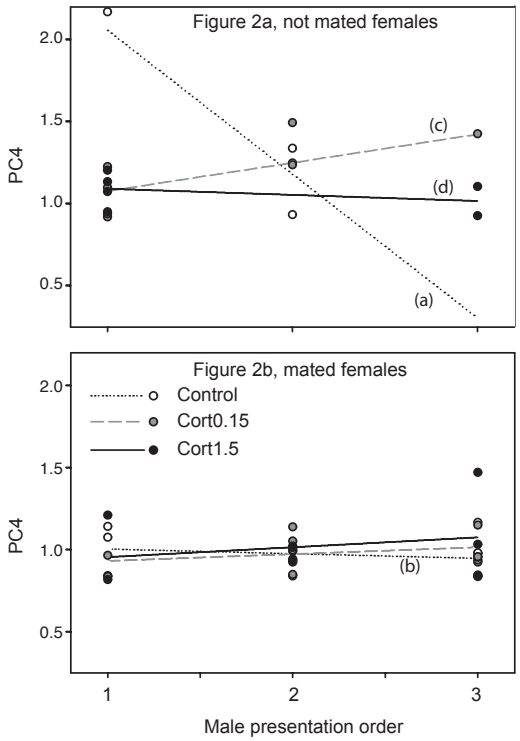

Figure 6. 2: Effects of male corticosterone treatment and male presentation order on female interest in interacting with males (represented by PC4*) of (*a*) not mated and *(b)* already mated females. Different shadings (empty = Control, grey = Cort0.15, black = Cort1.5) and different lines (black dotted for Control, grey dashed for Cort0.15 and black continuous for Cort1.5) represent data and predictions of different male corticosterone treatments, respectively. Lower case letters reflect post-hoc contrasts. * Ln transformed.

Discussion

Previous studies have shown that corticosterone may have negative effects on mating and sexual selection (Moore and Miller 1984; DeNardo and Licht 1993; Leary *et al.* 2006; Roberts *et al.* 2007). Consequently, we predicted that males with lower corticosterone levels would be better quality males and thus preferred by females. The observed female behaviour is in line with this prediction given that not mated females showed more interest for Control males presented in the first place than for the other males (Figure 6. 2a). In addition, defensive female behaviour towards Control males was less marked than towards males with moderate corticosterone levels, and there was a tendency that it was smaller than towards males with high corticosterone levels. Interestingly, males with moderate corticosterone levels conducted more approaches towards the female than Cort1.5 and Control males, indicating increased interest (Figure 6. 3). This shows, on one hand, that the most interested males were those with moderate corticosterone levels, and that females preferred control males to corticosterone treated males. This suggests that females may have preferences for better quality males, *i.e.* control males, and may have defended themselves more against corticosterone treated

males, with more defence directed towards the more interested Cort0.15 males. This finding as well indicates that females can and do resist against males, which is congruent with previous studies suggesting that female resistance to males may play an important role in intersexual interactions in this species (Fitze *et al.* 2005; Fitze and Le Galliard 2008; Fitze *et al.* 2010).

We as well predicted that females might perform context dependent sexual selection, *i.e.* that sexual selection depends on the female mating status. Our results show that interest for control males differed between mated females and not mated females. In not mated females the interest towards control males was highest towards first presented males, and no presentation order effect existed in mated females where interest was generally lower (Figure 6. 2). This suggests that not mated females are choosier at the first presentation, while mated females are less interested, which is consistent with context-dependent mate choice. In addition, longer pre-copulations of mated females might indicate increased male interest but might also be interpreted as female resistance to mate. Already mated females may be more reluctant to mate given that they may try to improve on the previous male. As a consequence, it may be harder for a male to convince an already mated female, leading to increased pre-copulation duration. Interestingly, copulation duration did not depend on mating status, suggesting that the decision to mate is most important and that mating per-se is not mating status dependent. Our results thus suggest that females performed context dependent intersexual behaviours taking into account male corticosterone levels, male presentation order and their previous mating history. This is in line with previous studies (Cooper *et al.* 1993; Censky 1997; Fitze *et al.* 2010) showing that females select better quality males (*i.e.* males with lower corticosterone levels) for their first mating.

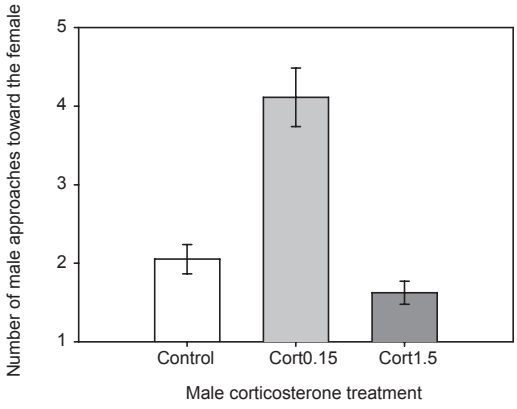

Figure 6. 3: Male approaches towards the female in relation to corticosterone treatment. Means ± SE are given and shading indicates the corticosterone treatment group (empty = Control, light grey = Cort0.15, dark grey = Cort1.5).

Female mating behaviour (PC1: number of pre-copulations and number of copulations) was not significantly affected by male corticosterone treatment, although we predicted that males with increased corticosterone levels would perform best-of-a-bad-job strategies to compensate for increased female reluctance (see Gonzalez-Jimena and Fitze 2012; *Chapter 5*). Male best-of-a-bad-job strategies may indeed explain the absence of corticosterone treatment effects on mating behaviour. The increased number of approaches towards the female conducted by males with moderate corticosterone levels actually suggests that males with moderate corticosterone levels may have compensated for increased female reluctance. Moreover, female interest for moderate corticosterone males increased with presentation order in not mated females, *i.e.* females that did not mate with control and high corticosterone males were more interested in the more active moderate corticosterone males. Not increasing female interest with respect to male presentation order may be the result of the associated risk of inverting into reproduction (see Le Galliard *et al.* 2008) despite that means a non-fertilized clutch (Bleu *et al.* 2011). In this case male activity may be key for breaking female reluctance (note that in high corticosterone males not mated females did not show changing interest with respect to male presentation order). This as well suggests that not mated females became less choosy once time went by and they were still not mated.

In summary, our experiment shows that male corticosterone levels affected both female and male intersexual behaviour but not mating behaviour. Approaching behaviour towards females was highest in moderate corticosterone males and females adjusted their defensive behaviour and their interest with respect to male corticosterone levels, male presentation order and their mating status. The latter shows that female inter-sexual behaviour depends on male glucocorticoid levels and thus that it is context-dependent. Previous findings suggested that moderate corticosterone levels might have positive effects on male intrasexual competition through increasing dominance status. However, in the present experiment, *i.e.* in the absence of male intrasexual competition, male moderate corticosterone levels did not positively affect female interest per se, nor did it reduce female defensive behaviour. In contrast, the results suggest that the increased number of approaches conducted towards females by moderate level males led to an increase of female interest with male presentation

order. Thus, males with moderate corticosterone levels may be most successful in unmated reluctant females that already had the opportunity to copulate with males, which may explain why moderate corticosterone males may on average be as successful as control males (*Chapter 5*). The results further support previous findings suggesting that male reproductive success may not be impaired by increased corticosterone levels (DeNardo and Licht 1993; Leary *et al.* 2006; Roberts *et al.* 2007). This suggests that dose-dependent corticosterone effects modulated context dependent female mate choice and intersexual male behaviour, and that they do not directly affect male reproductive success, given the existence of dose-dependent male compensatory strategies.

Specific Acknowledgments

We thank to Luis M. San-Jose, Miguel Peñalver Alcázar y Sergio Cánovas Fajardo for field and laboratory assistance. The capture and handling of lizards was conducted under the licenses provided by the Instituto Aragonés de Gestión Ambiental (Gobierno de Aragón). VGJ was supported by a PhD grant from the Spanish Ministry of Education and Science (FPU AP2006-01678) and PSF by a grant from the Spanish Ministry of Education and Science (Programa Ramón y Cajal, RYC-2003-006136). The project was financed by two grants from the Spanish Ministry of Education and Science (CGL2005-01187, CGL2008-01522).

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Chapter 7

7 General conclusions

Conclusiones

1 (*Objetivo 1*). El estrés afecta a la selección de microhábitat de la lagartija de turbera. En respuesta a factores estresantes de origen natural la lagartija de turbera cambia su comportamiento hasta llegar a regular de manera muy precisa la selección que hace del microhábitat, teniendo en cuenta las características intrínsecas del propio hábitat, el riesgo de posibles amenazas y la magnitud de las mismas.

2 (*Objetivo 2*). Cuando la lagartija de turbera se expone a varios factores estresantes y situaciones en la que aparece un conflicto, la selección de microhábitat se complica y pasa a depender de costes conductuales, específicos de la especie, y de interacciones complejas entre los anteriores factores, que pueden llevar a varias soluciones adecuadas. La precisa regulación de las respuestas conductuales sigue una jerarquía que va desde comportamientos de bajo coste en situaciones de bajo coste (sin conflicto presente) a comportamientos de alto coste en situaciones de alto coste (conflicto presente).

3 (*Objetivo 2*). Las lagartijas expuestas a olor de un depredador y de un individuo de su misma especie no difieren en cuanto a la selección de microhábitat, lo que sugiere que la competencia intraespecífica puede ser un modulador de la selección de microhábitat tan importante como el riesgo de depredación. Esto indica que la competencia intraespecífica en especies no territoriales debe ser más importante de lo que se creía.

4 (*Objetivo 3*). El comportamiento de apareamiento de los machos de lagartija de turbera no se ve inhibido por la presencia de niveles moderados o altos de corticosterona.

5 (*Objetivo 3*). En la lagartija de turbera la corticosterona tiene efectos dependientes de dosis en el comportamiento intrasexual relacionado directa o indirectamente con el apareamiento. A diferencia de los niveles control o elevados de corticosterona, los niveles moderados pueden ser beneficiosos durante las interacciones entre machos, dado que favorecen comportamientos que determinan el estado de dominancia.

6 (*Objetivo 4*). Los cambios en el comportamiento inducidos por la corticosterona dependen del contexto social, con respecto a la presencia o ausencia de competencia intrasexual:

6. a. (*Objetivo 4. a.*). En un contexto de competencia intrasexual las hembras de lagartija de turbera prefieren a los machos con niveles bajos de corticosterona. Los machos con niveles tanto moderados como altos de corticosterona realizan comportamientos adaptativos de compensación (estrategias de tipo "best-of-a-bad-job") para sobrellevar la reticencia de las hembras al apareamiento, demostrando mayor interés en interaccionar con ellas y realizando más intentos de cópula.

6. b. (*Objetivo 4. b.*). En un contexto libre de competencia intrasexual, sólo los machos de lagartija de turbera con niveles moderados de corticosterona desarrollan estrategias de compensación para sobrellevar la reticencia de las hembras al apareamiento, demostrando más interés por ellas que los machos con niveles control y elevados de corticosterona. Sin embargo, a diferencia del caso anterior, los machos con niveles moderados de corticosterona no aumentan el número de intentos de copula.

7 (*Objetivo 5*). El comportamiento intersexual de las hembras también depende de los niveles de corticosterona de los machos. Las hembras ajustan su comportamiento defensivo y su interés dependiendo del nivel de corticosterona de los machos. Además, el interés intersexual de las hembras depende del orden de presentación de los machos y del estado de apareamiento de las hembras. Esto sugiere que la selección de pareja dependiente de contexto por parte de las hembras debe ser un factor determinante de la selección sexual, y que los efectos de la corticosterona dependientes de dosis pueden modularla a través de sus efectos sobre comportamientos indirectamente relacionados con el apareamiento.

8. En un contexto más amplio, este estudio revela que el estrés y el incremento de los niveles basales de corticosterona (es decir, la respuesta

fisiológica al estrés) inducen respuestas conductuales que están determinadas de manera compleja por diferentes factores y contextos. A este respecto, el hecho de que el estrés o la respuesta fisiológica al estrés afecte positiva o negativamente a la eficacia biológica individual debe ser estudiado teniendo en cuenta los efectos de las interacciones potenciales entre aquellos factores. Es más, como se ha visto, la plasticidad conductual también tiene un papel importante en la modulación de las consecuencias del estrés y del incremento de los niveles de corticosterona. La relación entre comportamiento animal y estrés resulta al final en una intrincada red en la que el incremento de los niveles basales de corticosterona altera el comportamiento de los individuos sometidos a estrés, las interacciones entre estos y otros individuos de la población y entre los individuos sometidos a estrés y el ambiente que les rodea.

Conclusions

1 (*Goal 1*). Stress affects the common lizard's microhabitat selection. In the presence of natural stressors, common lizard's behavioural response results in a fine regulation of microhabitat selection that depends on intrinsic habitat characteristics, threat risk, threat magnitude, and complex trade-offs among them.

2 (*Goal 2*). When exposed to multiple stressors and trade-off situations, a common lizard's microhabitat selection becomes a complex task that depends on species-specific behavioural costs and complex interactions, potentially having several optimal solutions. The common lizard's fine-tuned responses follow a behavioural hierarchy ranging from low-cost behaviours conducted in low-cost situations (in the absence of a trade-off situation) to high cost behaviours conducted in high-cost situations (when a trade-off situation is present).

3 (*Goal 2*). Lizards exposed to predator and conspecific odour did not differ in microhabitat selection, suggesting that conspecific competition may be as an important modulator of microhabitat selection as predation risk. This indicates that conspecific competition is more important for non-territorial species than previously believed.

4 (*Goal 3*). Male copulation behaviour is not impaired by high or moderate corticosterone levels in the common lizard.

5 (*Goal 3*). Corticosterone has dose-dependent effects on common lizard intrasexual behaviours that are directly or indirectly related to mating. In this regard, in contrast to control or high corticosterone levels, moderate levels of corticosterone may be beneficial during male intrasexual interactions as they positively affect behaviours determining dominance.

6 (*Goal 4*). Corticosterone-induced behavioural changes depend on the social context in relation to the presence/absence of intrasexual-competition:

6. a. (*Goal 4. a.***).** In an intrasexual-competition-context, females prefer males with low corticosterone levels. Males with both moderate and high corticosterone levels perform adaptive compensatory behaviours (best-of-a-bad-job strategies) to circumvent female reluctance to mate by showing higher interest in interacting with females and by conducting more copulation attempts.

6. b. (*Goal 4. b.***).** In an intrasexual competition free context, only common lizard males with moderate corticosterone levels perform a compensatory strategy to circumvent female reluctance to mate by showing more interest for females than high level and control males. However, in an intrasexual competition free context, males with moderate corticosterone levels do not change their copulation attempts in order to cope with female reluctance to mate.

7 (*Goal 5***).** Female intersexual behaviour also depends on male corticosterone levels. Females adjust defensive behaviour and interest depending on male corticosterone levels. Moreover, intersexual female interest depends on male presentation order and female mating status. This suggests that context dependent female choice might be an important force driving sexual selection and that dose-dependent corticosterone effects may modulate it through its effects on behaviours indirectly related to mating.

8. In a broader context, this study reveals that stress and the increase in baseline corticosterone levels (*i.e.* physiological stress response) induce behavioural responses that are complexly determined by different factors and contexts. In this regard, whether stress or the physiological stress response negatively or positively affects individual fitness has to be studied at the light of the potential interactive effects of these factors. Moreover, as shown here, behavioural plasticity also plays an important role in modulating the consequences of stress and of the increase in corticosterone levels. The relationship between animal behaviour and stress finally result in an intricate network, where the increase in the baseline corticosterone levels alter the behaviour of stressed individuals, the interactions between those and other individuals in the population, and between stressed individuals and their surrounding environment.

Agradecimientos Generales

Supongo que todos nos vemos igual al llegar a este punto pero es realmente difícil dar las gracias a todos los que nos ayudan durante el largo y difícil proceso de acabar la tesis. Nos encontramos con mucha gente que comparte gran parte del camino o tan sólo una pequeña parte de él pero todos de una u otra manera terminan aportando algo así que espero no dejarme a nadie fuera.

Me gustaría empezar dando las gracias a mis compañeros de fatigas, a Luis por estar desde el primer día al pie del cañón para lo bueno y para hacer más llevadero lo malo. Miguel llegó algo después pero igualmente ha estado desde el primer momento ahí para todo lo que podía necesitar. Gracias a Merel, Cristina y Katleen por toda la ayuda que siempre han estado dispuestas a dar. No creo que haya compañeros de trabajo mejores y diga lo que diga de ellos me quedaré corta seguro. A Patrick no se cómo agradecerle la oportunidad que me brindó y la confianza que depositó en mi para llevar a cabo este proyecto ya que he podido aprender más de lo que podría imaginar durante todo el proceso. Quiero agradecer también a Rafa Zardoya todo su apoyo y útiles consejos, especialmente durante la última parte del proyecto. A Flora de Pablo que me diera a conocer el proyecto y también su implicación en la última parte del mismo. A José Luis Rubio, mi tutor, quiero agradecerle su ayuda con todos los tediosos trámites administrativos. A los demás compañeros del Museo también quiero darles las gracias sobre todo por todos esos "cafés" de los que siempre se saca algo interesante: Carmen, Borja, Goyo, y muchos más, ¡gracias a todos!

A la gente del IPE de Jaca quiero darles las gracias por prestarnos desde sus instalaciones hasta las cosas más insospechadas para poder llevar a cabo nuestros experimentos de "lagartijeros". Todos ellos han conseguido que el tiempo pasado en Jaca fuera mucho más ameno. Quiero hacer una mención especial a Sané y agradecerle de todo corazón su manera de ser. A Tere Suárez y los demás compañeros del CIB quiero agradecerles también todo lo que me enseñaron durante mi estancia allí.

Gracias también a todos los voluntarios que decidieron hacer sus prácticas con nosotros, sin duda todos tuvieron algo interesante que aportar a este proyecto a parte de mucha ayuda extra: Esperanza, Itziar, Sergio, Elisa, Jesús y Laura.

A mis compañeros de Los Sauces quiero agradecerles también todo su apoyo y especialmente todo lo que me han enseñado además de poner su biblioteca a mi disposición.

Por último pero no menos importante, quiero dar las gracias a todos esas personas que simplemente por estar cerca han hecho posible esta tesis. A mis padres nunca podré agradecerles lo suficiente que me dieran la oportunidad de estudiar lo que me gustaba, por raro que fuera, y por apoyarme siempre. A mi madre, mi hermana, mi prima y mis tías porque siempre están ahí. Gracias a Antón por todo su apoyo y su infinita paciencia aguantando todos los meses que he tenido que pasar fuera y mi monotemática conversación sobre la tesis. Gracias a mis abuelos que no van a poder ver el final de lo que empecé con su siempre incondicional apoyo. A todos Arias-Royo quiero darles las gracias por ver siempre el lado positivo. A mis amigos quiero darles también las gracias por tener paciencia conmigo y no darme por perdida a pesar de que siempre estaba liada con la tesis. Gracias a Elisa, las Marías, Cris, Dani, Carmen y los demás. A Vivi quiero agradecerle tantas cosas que no sabría por donde empezar pero sobre todo quiero darle las gracias por su optimismo, bondad, valor y fuerza, que han sido y serán siempre un ejemplo para todos los que la conocíamos.

Abbreviations

aa: Aminoacid

ACTH: Adrenocorticotropic hormone

C: Lizards exposed to no additional odour (Chapter 4).

CNS: Central Nervous System

Cort0.15: Lizards treated with corticosterone in order to produce a moderate corticosterone elevation (Chapters 5 and 6).

Cort1.5: Lizards treated with corticosterone in order to produce a high corticosterone elevation (Chapters 5 and 6).

CRH: Corticotropin-releasing hormone

F: Lizards exposed to adult female *Lacerta vivipara* odour (Chapter 4).
FA: Fatty acids

FSH: Follicle-stimulating hormone

GC: Glucocorticoids

GnRH: Gonadotropin-releasing hormone

HPA-axis: Hypothalamic-pituitary-adrenal axis

HPG-axis: Hypothalamic-pituitary-gonadal axis

HT: Habitat type

LH: Luteinizing hormone

M: Lizards exposed to adult male *Lacerta vivipara* odour (Chapter 4).

MC: Mineralocorticoids

P: Lizards exposed to adult predator odour (Chapter 4).

PC: Principal component

PCA: Principal component analysis

PNS: Peripheral Nervous System

POMC: Pro-opiomelanocortin

PT: Peripheral tissues

SNS: Sympathetic nervous system

SVL: Snout-vent length

T_b: Body temperature

TM: Threat magnitude

TR: Threat risk